(54) Title: PARATHYROID HORMONE ANTIBODIES AND RELATED METHODS
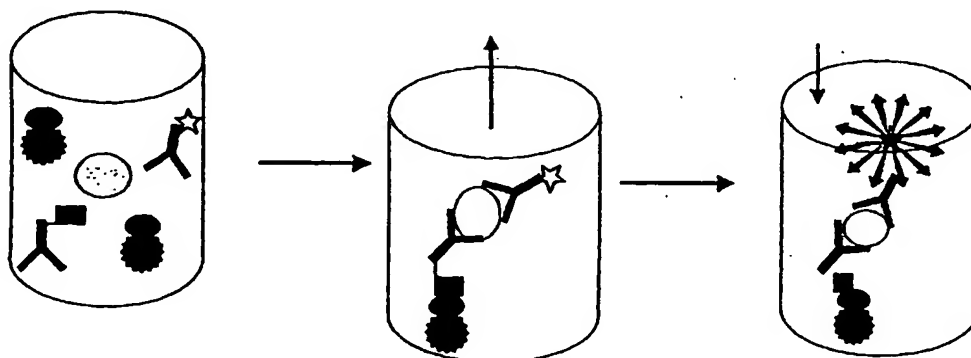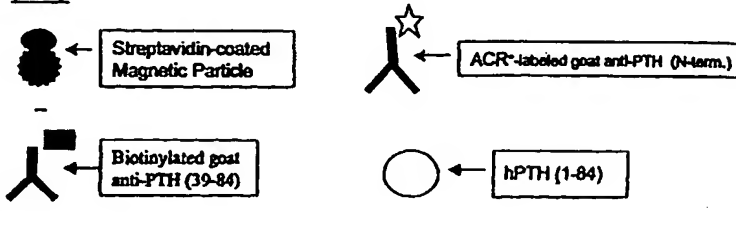
(57) Abstract: Methods of preparing antibodies that recognize and bind three-dimensional epitopes of antigens are disclosed. The methods are particularly useful for preparing antibodies that bind the bioactive, three-dimensional amino terminus of parathyroid hormone. The antibodies so produced are used in diagnostic and therapeutic applications.

PARATHYROID HORMONE ANTIBODIES AND RELATED METHODS

SUMMARY OF THE INVENTION

5   The present invention provides antibodies that specifically
     recognize three-dimensional epitopes of proteins and methods
     for producing and using same. In one embodiment, methods for
     producing antibodies that recognize and bind the bioactive,
     three-dimensional epitope of parathyroid hormone are

10   disclosed. In a preferred embodiment, the methods produce
     antibodies that recognize and bind the first thirteen amino
     acids of human parathyroid hormone. In a more preferred
     embodiment, the antibodies of the invention are substantially
     not crossreactive with non-bioactive parathyroid hormone.

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     The invention also provides an antibody (monoclonal or
     polyclonal), and a purified preparation of an antibody, which
     is capable of forming an immune complex with a three-
     dimensional epitope of an antigen (preferably a parathyroid

·20 hormone, such as a human parathyroid hormone) such antibody
     being generated by using as an antigen, parathyroid hormone,
     or a variant thereof. This antibody is preferably capable of
     neutralizing (i.e., partially or completely inhibiting) a
     biological activity of the parathyroid hormone (i.e., a

25   component of one of the cascades naturally triggered by the
     parathyroid hormone binding to its receptor). In preferred
     embodiments, the antibody of the invention is capable of
     forming an immune complex with parathyroid hormone receptor
     and is capable of neutralizing a biological activity of the

30   PTH receptor (i.e. adenylate cyclase activation or
     phospholipase C stimulation).

     Also within the invention is a therapeutic composition
     including, in a pharmaceutically-acceptable carrier, an

antibody to parathyroid hormone, or a derivative thereof.
These therapeutic compositions provide a means for treating
various disorders characterized by overstimulation of
parathyroid hormone receptors by a parathyroid hormone. These

5   antibodies are useful as diagnostics, such as for
distinguishing those cases of hypercalcemia related to PTH
from those which are not.

The antibody of the present invention recognizes any of the

10   above-mentioned peptides, for example human parathyroid
hormone.

In one embodiment, the antibody recognizes an amino acid
sequence from Ser at position 1 to Leu at position 13 of SEQ

15   ID No. 1 or a portion, or portions, included in the amino acid
sequence.

In one embodiment, the antibody is a polyclonal antibody or a
monoclonal antibody.

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The method for measuring parathyroid hormone or a variant
thereof according to the present invention includes the steps
of: incubating a mixture of a sample and a first antibody
recognizing the parathyroid hormone, or variant thereof;

25   adding a labeled second antibody recognizing the parathyroid
hormone, or variant thereof, to the mixture, followed by
further incubation; and detecting the resulting antigen-
antibody complex in the mixture.

30   Alternatively, the method for measuring parathyroid hormone or
a variant thereof according to the present invention includes
the steps of: incubating a mixture of a sample, a first
antibody recognizing the parathyroid hormone, or variant
thereof, and a labeled second antibody recognizing the

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parathyroid hormone, or variant thereof; and detecting the resulting antigen-antibody complex in the mixture.

The immunological assay for a peptide of the present invention
5   comprises the steps of: incubating a sample including any of the above-mentioned peptides with any of the above-mentioned antibodies under conditions for forming an antigen-antibody complex; and quantifying the antigen-antibody complex.

10   The kit for an immunological assay of the bioactive forms of parathyroid hormone includes any of the above-mentioned antibodies.

Any feature or combination of features described herein are
15   included within the scope of the present invention provided that the features included in any such combination are not mutually inconsistent as will be apparent from the context, this specification, and the knowledge of one of ordinary skill in the art.
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Additional advantages and aspects of the present invention are apparent in the following detailed description and claims.

BRIEF DESCRIPTION OF THE FIGURES
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FIG. 1 is a schematic illustration of the procedures used according to the invention.

FIG. 2 depicts graphs of observed PTH concentration
30   (picomoles) versus spiked PTH (picomoles). Spiked PTH is defined as the addition of the specified amount of PTH in picomoles into the assay reaction. FIG. 2A illustrates the Nichols Advantage Intact-PTH assay. FIG. 2B illustrates the bio-intact PTH assay using the antibodies of the invention.

FIG. 3 is a graph of PTH concentration (picomoles) versus fractionation number for bio-intact PTH (using the antibodies of the invention) and intact PTH (using the Nichols Advantage

5 Intact PTH assay). Note the absence of the peak corresponding to the fragment, PTH_{7-84}, using the antibodies of the invention (bio-intact PTH).

FIG. 4 is a graph of observed PTH concentration (picomoles) as

10 a function of "competitor" concentration (picomoles). The competitors are PTH peptides consisting of amino acids 1-34, 2-34, 3-34, 4-34, and 5-34 of PTH.

FIG. 5 is a graph of percent dose coefficient of variation

15 versus bio-intact PTH concentration (picograms/mL).

FIG. 6 is a graph of bio-intact PTH concentration (picograms/mL) versus Nichols Advantage intact-PTH concentration (picograms/mL) for "normal" subjects.

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FIG. 7 is a graph of bio-intact PTH concentration (picograms/mL) versus Nichols Advantage intact-PTH concentration (picograms/mL) for subjects with impaired renal function.

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FIG. 8 is a graph of bio-intact PTH concentration (picograms/mL) versus Nichols Advantage intact-PTH concentration (picograms/mL) for subjects with chronic renal failure.

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FIG. 9 is a graph of manual immunoradiometric assay PTH concentration (picograms/mL) versus automated ICMA PTH concentration (picograms/mL) for bio-intact PTH. ICMA is defined as Immuno Chemiluminescence Metric Assay. Bio-intact

PTH refers to the assay of the invention in which PTH is measured with the antibodies of the invention (e.g., anti-PTH$_{1-13}$) as the detection (tag) antibody, and with anti-PTH$_{38-84}$ as the capture antibody.

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FIG. 10 is a graph illustrating the ratio of PTH to anti-PTH$_{1-13}$ antibodies as a function of the inhibitory PTH peptide. "1-6" indicates the use of a peptide consisting of amino acids 1-6 of PTH (SEQ ID NO: 1); "7-13" indicates the use of a peptide

10 consisting of amino acids 7-13 of PTH (SEQ ID NO: 1); and "1-13" indicates the use of a peptide consisting of amino acids 1-13 of PTH (SEQ ID NO: 1).

FIG. 11 is a graph illustrating the ratio of PTH to anti-PTH$_{1-13}$

15 antibodies as a function of the inhibitory PTH peptide. "1-34" indicates the use of a peptide consisting of amino acids 1-34 of PTH (SEQ ID NO: 1); "2-34" indicates the use of a peptide consisting of amino acids 2-34 of PTH (SEQ ID NO: 1); "3-34" indicates the use of a peptide consisting of amino

20 acids 3-34 of PTH (SEQ ID NO: 1); "4-34" indicates the use of a peptide consisting of amino acids 4-34 of PTH (SEQ ID NO: 1); and "5-34" indicates the use of a peptide consisting of amino acids 5-34 of PTH (SEQ ID NO: 1).

25 FIG. 12 is a graph illustrating the ratio of PTH to anti-PTH$_{1-13}$ antibodies as a function of the inhibitory PTH peptide. "1-6" indicates the use of a peptide consisting of amino acids 1-6 of PTH (SEQ ID NO: 1); "1-7" indicates the use of a peptide consisting of amino acids 1-7 of PTH (SEQ ID NO: 1); "1-8"

30 indicates the use of a peptide consisting of amino acids 1-8 of PTH (SEQ ID NO: 1); "1-9" indicates the use of a peptide consisting of amino acids 1-9 of PTH (SEQ ID NO: 1); "1-10" indicates the use of a peptide consisting of amino acids 1-10 of PTH (SEQ ID NO: 1); "1-11" indicates the use of a peptide

consisting of amino acids 1-11 of PTH (SEQ ID NO: 1); "1-12"
indicates the use of a peptide consisting of amino acids 1-12
of PTH (SEQ ID NO: 1); and "1-13" indicates the use of a
peptide consisting of amino acids 1-13 of PTH (SEQ ID NO: 1).

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FIG. 13 is a graph illustrating the ratio of PTH to anti-PTH_{1-13}
antibodies as a function of the inhibitory PTH peptide. "1-
13" indicates the use of a peptide consisting of amino acids
1-13 of PTH (SEQ ID NO: 1); "1-38" indicates the use of a
10 peptide consisting of amino acids 1-38 of PTH (SEQ ID NO: 1);
"1-34" indicates the use of a peptide consisting of amino
acids 1-34 of PTH (SEQ ID NO: 1); "1-84" indicates the use of
a peptide consisting of amino acids 1-84 of PTH (SEQ ID NO:
1); and "1-13" indicates the use of a peptide consisting of
15 amino acids 1-13 of PTH (SEQ ID NO: 1).

FIG. 14 depicts graphs of PTH concentration (picomoles) as a
function of retention time (minutes). FIG. 14A depicts the
HPLC measurements for PTH standards (PTH_{1-84}; PTH_{7-84}; PTH_{1-38};
20 and PTH_{1-34}). FIG. 14B depicts the HPLC measurements for a
"high" PTH group (e.g., greater than 200pg/ml). Data are
shown for "intact PTH" (closed diamonds); PTH_{1-84} (closed
squares); and PTH_{1-38} (triangles).

25 FIG. 15 depicts graphs of PTH concentration (picomoles) as a
function of retention time (minutes). FIG. 15A depicts the
HPLC measurements for a "normal" PTH group. Data are shown
for "intact PTH" (closed diamonds); PTH_{1-84} (closed squares);
and PTH_{1-38} (triangles). FIG. 15B depicts the HPLC measurements
30 for a "high" PTH group (e.g., greater than 500pg/ml). Data
are shown for "intact PTH" (closed diamonds); PTH_{1-84} (closed
squares); and PTH_{1-38} (triangles).

FIG. 16 depicts graphs of PTH concentration (picomoles) as a function of retention time (minutes). FIG. 16A depicts the HPLC measurements for samples without protease inhibitors. FIG. 16B depicts the HPLC measurements for samples with

5   protease inhibitors. Data are shown for "intact PTH" (closed diamonds); PTH_{1-84} (closed squares); and PTH_{1-38} (triangles).

FIG. 17 depicts graphs of PTH concentration (picomoles) as a function of retention time (minutes). FIG. 17A depicts the

10   HPLC measurements for subject K. FIG. 17B depicts the HPLC measurements for subject G. FIG. 17C depicts the HPLC measurements for subject P. Subjects K, G, and P all had chronic renal failure

15   FIG. 18 is a graph of observed PTH concentration (picomoles) as a function of "competitor" concentration (picomoles). The competitors are PTH peptides consisting of amino acids 1-84, 7-84, 7-13, 1-6, 1-34, 13-34, and 1-13 of PTH.

20   DETAILED DESCRIPTION OF THE INVENTION

Although the following description discloses antibodies that recognize and bind bioactive parathyroid hormone, and in particular, human parathyroid hormone, and methods of making

25   and using the same, the description should not be construed to be limited to only parathyroid hormone. In that regard, the methods and uses disclosed herein, can be practiced with any polypeptide in which the bioactivity of the polypeptide is related, at least in part, to the polypeptide's, or a portion

30   thereof, tertiary structure.

I.   Definitions

Unless defined otherwise, all technical and scientific terms
used herein have the same meaning as commonly understood by

5   those of ordinary skill in the art to which this invention
belongs.   For purposes of the present invention, the following
terms are defined below.

As used herein, the terms "PTH" and "parathyroid hormone" are

10   used interchangeably.   Parathyroid hormone regulates calcium
absorption directly in the kidney and bone, and indirectly in
the intestine.   Mature parathyroid hormone is synthesized in
vivo by cleavage of the signal sequence of "preproPTH", and
the subsequent cleavage of the "pro" sequence from "proPTH".

15   Parathyroid hormone, as used herein, encompasses parathyroid
hormone from any animal capable of making parathyroid hormone,
including, but not limited to, humans, non-human primates,
horses, dogs, cats, goats, and rodents.   In addition,
parathyroid hormone encompasses parathyroid hormone variants,

20   discussed herein, that include recombinantly synthesized
parathyroid hormone expressed in organisms that naturally do
not express parathyroid hormone.

As used herein, the terms "hPTH", "human PTH", and "human parathyroid hormone" are used interchangeably. In addition, hPTH is encompassed by the terms PTH or parathyroid hormone. Human PTH consists essentially of 84 alpha-amino acid residues
5   arranged by amide linkage in the sequence (SEQ ID NO:1) identified below:

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       Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly
       Lys His Leu Asn Ser Met Glu Arg Val Glu Trp Leu
10     Arg Lys Lys Leu Gln Asp Val His Asn Phe Val Ala
       Leu Gly Ala Pro Leu Ala Pro Arg Asp Ala Gly Ser
       Gln Arg Pro Arg Lys Lys Glu Asp Asn Val Leu Val
       Glu Ser His Glu Lys Ser Leu Gly Glu Ala Asp Lys
       Ala Asp Val Asn Val Leu Thr Lys Ala Lys Ser Gln
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Human PTH can be synthesized in vivo, or synthetically using standard techniques known in the art.

Persons skilled in the art will appreciate that variations in
20   the structure or sequence of PTH may occur. As used herein, a "variant" of PTH is defined as a polypeptide that possesses biological activity that is similar, or substantially similar, to a biological activity of PTH. A molecule is said to be "substantially similar" to another molecule if both molecules
25   have substantially similar structures, or if both molecules possess a similar biological activity. For example, a biological activity of PTH includes the binding of PTH to a receptor for PTH and preventing the subsequent binding or action of PTH at that receptor. Another biological activity
30   of PTH includes regulation of adenylate cyclase activity. Variants of PTH include analogs, fragments, or extensions, of PTH. Variants of PTH include naturally occurring PTH and recombinantly synthesized PTH. For example, a "variant" of PTH may have one or more amino acid substitutions. An amino

acid substitution may be conservative or non-conservative, as
is well understood in the art. A conservative substitution
refers to a substitution of one amino acid with another amino
acid without affecting the biological activity, or tertiary

5   (e.g., three-dimensional) structure, of the protein. In
addition, variants of PTH include PTH molecules that have
modified amino acid side chains, as is well known in the art.
Thus, if a molecule possesses at least one biological activity
that is similar to an activity of PTH, it is considered a

10   "variant" of PTH. For example, variants include peptides or
polypeptides that essentially have the sequence of PTH amino
acids in the 1-13 region. Variants also include such peptides
with approximately 1 or 2 amino acid substitutions. Further
examples of variants of hPTH conist or comprise the following

15   sequences (SEQ ID NOs: 2-8):

           Ala-Val-Ser-Glu-Ile-Gln-Phe-Met-His-Asn-Leu-Gly-Lys

           Ala-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys

20

           Ser-Val-Ser-Glu-Ile-Gln-Leu-Met-His-Asn-Leu-Gly-Lys

           Ser-Val-Ser-Glu-Ile-Gln-Leu-Cys-His-Asn-Leu-Gly-Lys

25   Ala-Val-Ser-Glu-Ile-Gln-Phe-Cys-His-Asn-Leu-Gly-Lys

           Ala-Val-Ser-Glu-Ile-Gln-Leu-Cys-His-Asn-Leu-Gly-Lys

           Ser-Val-Ser-Glu-Ile-Gln-Leu-Cys-His-Asn-Leu-Gly-Lys

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As used herein, "bioactive PTH" or "bioactive hPTH" refers to
a PTH, or hPTH, polypeptide that has at least one biological
activity of PTH. For example, PTH is considered to be
bioactive when the polypeptide is able to regulate adenylate

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cyclase activity. In one embodiment, PTH regulates adenylate
cyclase activity by binding to its receptor. Other biological
activities are also used to define bioactive PTH. Examples of
bioactive hPTH include PTH variants that comprise at least

5 amino acids 1-13 of SEQ ID NO:1. A further example of
bioactive PTH includes amino acids 1-34 of SEQ ID NO: 1. A
full length PTH molecule, such as amino acids 1-84 of SEQ ID
NO: 1, is also a bioactive PTH.

10 As used herein, "antibody" refers to a polypeptide
substantially encoded by an immunoglobulin gene or
immunoglobulin genes, or fragments thereof, which specifically
recognize and bind an antigen. The recognized immunoglobulin
genes include the kappa, lambda, alpha, gamma, delta, epsilon

15 and mu constant region genes, as well as the immunoglobulin
variable region genes. Antibodies include fragments, such as
Fab', F(ab)$_2$, Fabc, and Fv fragments. The term "antibody," as
used herein, also includes antibody fragments either produced
by the modification of whole antibodies or those synthesized

20 de novo using recombinant DNA methodologies, and further
includes "humanized" antibodies made by now conventional
techniques.

An antibody "specifically binds to" or "is immunoreactive
25 with" a protein when the antibody functions in a binding
reaction with the protein. The binding of the antibody to the
protein permits determination of the presence of the protein
in a sample in the presence of a heterogeneous population of
proteins and other agents. Thus, under designated immunoassay
30 conditions, the specified antibodies bind preferentially to a
particular protein and do not significantly bind to other
proteins present in the sample. Specific binding to a protein
under such conditions requires an antibody that is selected
for specificity for a particular protein. Several methods for

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determining whether or not a peptide is immunoreactive with an antibody are known in the art. Immuno chemiluminescence metric assays (ICMA), enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA) are some examples.

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An antibody is "selective for" bioactive PTH when the antibody preferentially binds to bioactive forms of PTH compared to non-bioactive forms of PTH. In a preferred embodiment, an antibody is selective for bioactive PTH when it binds to

10 bioactive PTH at least 10 times more preferentially than non-bioactive PTH. In a more preferred embodiment, the antibody selective for bioactive PTH binds at least 100 times more preferentially than non-bioactive PTH. In another preferred embodiment, the antibody selective for bioactive PTH binds at

15 least 200 times more preferentially than non-bioactive PTH. In yet another preferred embodiment, the antibody selective for bioactive PTH binds at least 500 times more preferentially than non-bioactive PTH. In an even more preferred embodiment, the antibody selective for bioactive PTH binds at least 1000

20 times more preferentially than non-bioactive PTH.

A "label" is a composition detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include fluorescent dyes,

25 chemiluminescent compounds, radioisotopes, electron-dense reagents, enzymes, colored particles, biotin, or dioxigenin. A label often generates a measurable signal, such as radioactivity, fluorescent light, color, or enzyme activity, which can be used to quantitate the amount of bound label.

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Examples of chemiluminescent compounds include luciferin, a luminol derivative, pyrogallol, isoluminol, aequorin, cyclic arylhydrazides, dioxetanes, rhodium chelates (electrochemiluminescent), oxalate esters,

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thermochemiluminescent labels, acridinium and the like. These
labels may be attached to a protein, for example an anti-PTH
antibody, using techniques well known in the art. (See U.S.
Patent No. 5,284,952, the disclosure of which is incorporated
5   in its entirety herein by reference.) In one embodiment, a
detection antibody, such as an anti-hPTH$_{1-13}$ antibody, may be
labeled with an acridinium by employing the methods found in
U.S. Patent Nos. 5,284,952, 5,110,932, and 5,338,847, the
disclosures of which are incorporated in their entirety herein
10 by reference.

Examples of the fluorescent material to be used for labeling
include fluorescein, fluorescamine, fluorescein
isothiocyanate, umbelliferone, rhodamine, Texas red dyes,
15 pthalocyanines, coumarin, squaraine, anthracene, erythrosine,
europium chelates and the like.

Examples of radioactive isotopes to be used for labeling
include ^{14}C, ^{3}H, ^{32}P, ^{18}F or ^{125}I.
20

Exemplary enzymes which have been developed and can be used in
assays of the invention are those described in U.S. Pat. Nos.
3,654,090; 3,791,932; 3,839,153; 3,850,752; U.S. Pat. No.
3,817,837; 3,879,262; Journal of Immunological Methods 1:
25 247(1972); and the Journal of Immunology 109:129(1972), the
disclosures of which are incorporated in their entirety herein
by reference. Other examples of enzymes include, but are not
limited to, alkaline phosphatase, beta galactosidase,
horseradish peroxidase, gluconidase, phosphatase, peptidase,
30 alkaline phosphatase and the like. Co-enzymes useful in this
invention include molecules and/or proteins which facilitate
an enzyme to catalyze a reactant to produce a detectable
product, for example light. A co-enzyme may include, without
limitation, FAD and NAD. In one embodiment, an anti-PTH may

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be labeled with a NAD. See for example, U.S. Patent
4,380,580, the disclosure of which is incorporated in its
entirety herein by reference.

5   Examples of colored particles include colloidal gold, or blue
latex. In certain embodiments, labels are coupled to the
antibodies of the invention to permit determination of the
presence of bioactive forms of parathyroid hormone.

10   Other labels may include a non-active precursor of a
spectrophotometrically-active substance (British Pat. No.
1,392,403 and French Pat. No. 2,201,299, which patents
correspond to U.S. Pat. No. 3,880,934) and electron spin
resonance moieties (U.S. Pat. No. 3,850,578).

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The terms "isolated", "purified", or "biologically pure" refer
to material that is at least partially separated from, and
that is often substantially, or essentially, free from
components which normally accompany it as found in its native
20   state. Purity and homogeneity are typically determined using
analytical chemistry techniques such as polyacrylamide gel
electrophoresis or high performance liquid chromatography, as
is well understood in the art. Generally, an isolated
antibody will comprise more than 80% of all macromolecular
25   species present in the preparation. Preferably, the antibody
is purified to represent greater than 90% of all
macromolecular species present. More preferably, the antibody
is purified to greater than 95%, and even more preferably, the
antibody is purified to essential homogeneity, wherein other
30   macromolecular species are not detected by conventional
techniques.

The term "immunoassay" is an assay that utilizes an antibody
to specifically bind an analyte. The immunoassay is

characterized by the use of specific binding properties of a
particular antibody to isolate, target, and/or quantify the
analyte.

5   The inventor of the present invention conducted numerous
studies for the purpose of rapidly, readily, and precisely
measuring bioactive PTH, specifically hPTH. As a result, he
produced an antibody that specifically recognizes bioactive
forms of hPTH. The inventor has constructed immunoassays
10   using combinations of antibodies, each recognizing different
epitopes of the hPTH.

        II.   Compositions of the invention

15   The antibodies of the invention recognize and bind three-
dimensional epitopes of proteins. In particular, the
antibodies specifically recognize bioactive forms of PTH. One
example of a protein that binds to the antibodies of the
invention is human PTH.
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        The antibodies of the invention have been deposited with the
American Type Culture Collection (10801 University Blvd.,
Manassas, VA 20110-2209, USA) on July 3, 2001, and have been
designated Accession No. PTA-3496. The deposit was viable at
25   the time of deposit and will be replaced by Applicants should
they become non-viable.

        The deposit will be made available to the Commissioner during
pendency of the application under the terms of 37 CFR 1.14 and
30   35 USC § 122, and will be maintained in the depository for a
period of 30 years or 5 years after the last request,
whichever is longer.

15

The preferred antibodies of the invention recognize and bind
bioactive forms of hPTH (bioactive hPTH). Without wishing to
limit the invention to any theory or mechanism of operation,
it is believed that the first amino acid (Ser at position 1 of

5 SEQ ID NO: 1) along with the intact helix structure of the
amino terminus (N-terminal region) of hPTH must be present in
order for hPTH to be bioactive. Furthermore, the bioactive N-
terminal region of hPTH is thought to be a three dimensional
helical structure consisting of thirteen amino acids (amino

10 acids 1-13 of SEQ ID NO: 1). The antibodies of the invention
recognize and bind to a three-dimensional epitope of the N-
terminal region. In one embodiment, the epitope comprises all
of the thirteen amino acids of hPTH (amino acids 1-13 of SEQ
ID NO: 1). In other embodiments, the epitopes comprise a

15 combination of the amino acids within the first thirteen amino
acids of hPTH. For example, the epitopes may comprise a
combination of amino acids 3, 4, and 5; 2, 4, and 6; 8, 10,
and 13; or mixtures thereof.

20 In one embodiment, antibodies of the present invention
recognize and bind at least one amino acid within the first
thirteen amino acids and at least one additional amino acid
located within the region consisting of amino acids 14 to 84,
preferably 13 to 34. For example, antibodies of the present

25 invention may recognize and bind to an epitope comprising
amino acids 3, 7, and 14; 4, 8, and 33; or 5, 14, and 15.

In one embodiment of the invention, the antibodies of the
invention are polyclonal antibodies.

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In another embodiment, the antibodies of the invention are
monoclonal antibodies.

In one embodiment of the invention, the antibody recognizes
and binds an epitope within the first thirteen amino acids of
PTH, or hPTH, and does not bind an epitope of PTH, or hPTH,
beyond the first thirteen amino acids. In a preferred

5 embodiment, the antibody recognizes and binds an epitope
within the first seven amino acids of PTH, or hPTH.

III. Methods of making the compositions of the invention

10 Parathyroid hormone or variants thereof, may be used to
generate antibodies by any conventional method well known to
those skilled in the art, including those which generate
polyclonal antibodies and those which generate monoclonal
antibodies.

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The polyclonal antibodies of the invention may be produced by
immunizing animals with intact PTH, variants thereof, or
mixtures thereof, in an emulsion, such as Freund's complete
adjuvant. Examples of animals used to produce antibodies

20 include, but are not limited to, mice, rats, rabbits, or
goats. Intact PTH may be defined as the full length PTH
molecule, for example, the PTH polypeptide after the "pre" and
"pro" sequences have been cleaved. In embodiments where human
PTH is employed, the intact hPTH has the amino acid sequence

25 depicted in SEQ ID NO: 1. In a preferred embodiment, the
intact PTH is preferably linked to a carrier protein, such as
keyhole limpet hemocyanin (KLH). Other examples of carrier
proteins include bovine serum albumin (BSA), hemocyanin, and
bovine thioglobulin (BTG). In certain embodiments, the

30 carrier protein is linked to the antigen of interest (e.g.,
PTH) with glutaraldehyde, as described herein. Other methods
are well known in the art.

After the initial immunization, the animals receive one or
more additional immunization boosts of substantially pure,
intact bioactive PTH, variants thereof, or mixtures thereof.
The immunizations can be administered intraperitoneally,
5   subcutaneously, or intravenously. Thus, the antibodies to
surface conformationally correct epitopes are elevated above
the large spectrum of antibodies to internal epitopes of PTH.

The animal's immune response to the immunogen preparation may
10 be monitored by taking test bleeds and determining the titer
of reactivity to the polypeptide of interest. When
approximately high titers of antibody to the immunogen are
obtained, blood is collected from the animal and antisera are
recovered.

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In one embodiment, the antibodies are isolated by exposing the
sera to antibody affinity purification columns. Other
techniques of isolating the antibodies are well known in the
art. The affinity columns are constructed by linking peptides
20 of PTH, preferably hPTH, to a solid phase of the columns. In
preferred embodiments of the invention, the columns include
hPTH peptides consisting of amino acids 1-13 ($hPTH_{1-13}$), 13-34
($hPTH_{13-34}$), and 39-84 ($hPTH_{39-84}$) of hPTH. Other peptides, or
combinations thereof, could be utilized to isolate the
25 antibodies of the invention.

As persons skilled in the art will readily understand, the
peptides $hPTH_{1-13}$ will specifically bind to antibodies that
recognize epitopes of hPTH within the first thirteen amino
30 acids of hPTH. Similarly, peptides $hPTH_{13-34}$ will specifically
bind to antibodies that recognize epitopes of hPTH within the
amino acids 13-34 of hPTH. Also, peptides $hPTH_{39-84}$ will bind
to antibodies that recognize epitopes of hPTH within the amino
acids 39-84 of hPTH.

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Thus, by way of the foregoing method, the antibodies that
specifically bind to an epitope within the first thirteen
amino acids of hPTH (anti-hPTH$_{1-13}$ antibodies) are selectively

5   isolated. In addition, the epitopes recognized by the anti-
hPTH$_{1-13}$ antibodies can include any combination of amino acids
within the first thirteen amino acids. In one embodiment, the
antibodies recognize an epitope consisting of the entire
thirteen amino acids of hPTH (i.e., amino acids 1-13 of SEQ ID

10   NO: 1).

As persons skilled in the art will understand, the order in
which the antibody-containing sera are exposed to the peptide-
coupled affinity columns is not determinative in practicing

15   the invention. However, in preferred embodiments, it is
desired to first expose the sera to a column containing the
hPTH$_{39-84}$ peptides, then to expose the resulting sera (e.g., the
sera without the antibodies that bind hPTH$_{39-84}$) to a column
containing hPTH$_{13-34}$ peptides, and subsequently exposing the

20   resulting sera (e.g., the sera without hPTH$_{39-84}$ and hPTH$_{13-34}$
antibodies) to a column containing hPTH$_{1-13}$ peptides.

Monoclonal antibodies that recognize and bind bioactive PTH
are also provided. Monoclonal antibodies can be produced

25   using conventional methods known in the art. See, for
example, Kohler and Milstein, (1975) Nature, 256:495-97.
Briefly, animals, such as mice, are injected with PTH
preferably coupled to a carrier protein as described above.
The animals are boosted with one or more pure PTH injections,

30   and are hyperimmunized by an intravenous (IV) booster 3 days
before fusion. Spleen cells from the mice are isolated and
are fused by standard methods to myeloma cells. Hybridomas
are selected in standard hypoxanthine/aminopterin/thymine
(HAT) medium, according to standard methods. Hybridomas

19

secreting antibodies which recognize bioactive PTH are
identified, cultured, and subcloned using standard
immunological techniques.

5   Frequently, the antibodies will be labeled by joining, either
covalently or non-covalently, a substance which provides for a
detectable signal. In one embodiment of the invention, the
labels are chemiluminescent compounds. A preferred
chemiluminescent compound is an acridinium ester.

10

III. Methods of using the composition of the invention

A.   Immunoassays

15 The immunological assays of the present invention can be used
for quantifying an antigen in a sample. Examples of various
assays and related reagents and parameters are provided in
U.S. Ser. No. 09/761,969, filed January 16, 2001, the entire
contents of which are incorporated by reference.

20

One example of an immunoassay is a "sandwich" immunoassay. In
the sandwich immunoassay, an antibody is immobilized on a
solid phase (capture antibody), incubated with an antigen, and
further incubated with an antibody with a detectable label
25   (detection antibody). In a preferred embodiment of the
invention, a PTH antibody that does not recognize the
bioactive portion of PTH is coupled to a solid phase, such as
magnetic particles, the antibody is incubated with a sample
containing PTH, and is further incubated with an antibody that
30   specifically binds bioactive PTH, coupled to a detectable
label. In a more preferred embodiment, a hPTH antibody that
does not recognize amino acids 1-13 of hPTH is coupled to
magnetic particles, the antibody is incubated with a sample
containing hPTH, and is further incubated with an antibody

20

that recognizes bioactive hPTH coupled to an acridinium ester.
In the sandwich immunoassay of the present invention, a
sample, the first antibody and the labeled second antibody may
be incubated simultaneously. Examples of suitable solid phase
5 substrates include, but are not limited to, microtiter plates,
beads, tubes, membranes, filter papers, or plastic cups.

Another example of the immunological assays include assays in
which an antigen labeled with a detectable label and an
10 unlabeled antigen are competitively reacted with an antibody.
In addition, an antigen can be immobilized on a solid phase,
incubated with a diluted antiserum or a purified antibody, and
further incubated with an antiimmunoglobulin labeled with a
detectable label, thereby obtaining a labeled binding
15 substance.

When the sandwich technique is used, two kinds of antibodies
against different epitopes of PTH are first prepared. One
antibody is labeled with a detectable label (detection
20 antibody) and the other antibody is allowed to bind to a solid
phase as a solid phase antibody or is made to be able to
specifically bind to a solid phase (capture antibody). These
antibodies are allowed to react with antigens in various
concentrations to form a plurality of antigen-antibody
25 complexes. Since the antigen-antibody complexes are bound to
solid phases, the solid phases are separated from the
complexes, and the amount of label in the solid phases is
measured. The relationship between the label and the
concentration of the antigen is plotted to obtain a standard
30 curve.

When a sample including an unknown concentration of antigen is
added to the reaction system, the concentration of the antigen
can be determined by applying the amount of label measured

21

after the reaction to the standard curve. Examples of samples
to be measured can be those containing PTH, or variants
thereof, and include plasma, serum, blood, urine, and the
like.

5

The sandwich immunoassay of the present invention includes a
sandwich radioimmunoassay (RIA), a sandwich enzymeimmunoassay
(EIA), a sandwich fluoroimmunoassay (FIA), a sandwich
chemiluminescence immunoassay (CLIA, ICMA), a sandwich

10   chemiluminescence-enzymeimmunoassay (CLEIA) and an
immunochromatographic method based on the sandwich assay.

The coupling of the capture antibody to the solid phase, and
the labeling of the detection antibody can be performed by any

15   method known to those skilled in the art. In a preferred
embodiment of the invention, the capture antibody is coupled
to biotin, and the magnetic particles are coated with
streptavidin. The biotin binds to the streptavidin, thereby
achieving the coupling of the capture antibody to the solid

20   phase.

B.   SCREENING ASSAYS

The present invention further provides assays for detecting

25   levels of PTH in a subject. In a preferred embodiment, the
assays provide methods for detecting abnormal levels of PTH in
a subject's blood.

These assays are particularly useful for screening test

30   compounds to determine their ability to regulate PTH activity.
In such cases, a test compound is added to the reaction system
and the effect of the test compound on the binding of
antibodies to PTH can be observed. Those compounds which
inhibit the binding of the antibodies of the invention to

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bioactive PTH can be considered as potential bioactive PTH
inhibitors and further as potential therapeutic agents for
treatment of conditions associated with abnormal PTH activity
or concentrations.

5

In one example, those antibodies that recognize PTH are
screened for their ability to compete with PTH for binding to
a PTH receptor. ·The antibody used may be from crude
antiserum, cell medium, or ascites, or in purified form.

10 Antibodies that reduce binding of the PTH analog to the PTH
receptor are classified as competitive; those which do not are
noncompetitive.

Compounds, including antibodies, may be screened for their
15 agonistic or antagonistic properties using cAMP accumulation,
intracellular calcium, and/or inositol phosphate assays.
Cyclic AMP accumulation can be measured by the assays, as
described above. A compound that competes with PTH for
binding to the PTH receptor, and that inhibits the effect of
20 PTH on cAMP accumulation, is considered a competitive PTH
antagonist. Conversely, a compound that does not compete for
PTH binding to the PTH receptor, but which still prevents PTH
activation of cAMP accumulation (presumably by blocking the
receptor activation site) is considered a non-competitive
25 antagonist. A compound that competes with PTH for binding to
the PTH receptor, and which stimulates cAMP accumulation in
the presence or absence of PTH, is a competitive agonist. A
compound that does not compete with PTH for binding to the PTH
receptor but which is still capable of stimulating cAMP
30 accumulation in the presence or absence of PTH, or which
stimulates a higher accumulation than that observed by PTH
alone, would be considered a non-competitive agonist.

23

C.   Diagnostic uses

The antibodies of the invention are useful for the diagnosis, classification, prognosis, and/or treatment of disorders which
5   may be characterized as related to the interaction between a cell receptor of the invention and its specific ligand. For example, some forms of hypercalcemia and hypocalcemia are related to the interaction between PTH and the PTH receptor(s). Hypercalcemia is a condition in which there is
10   an abnormal elevation in serum calcium level; it is often associated with other diseases, including hyperparathyroidism, osteoporosis, carcinomas of the breast, lung and prostrate, epidermoid cancers of the head and neck of the esophagus, multiple myeloma, and hypernephroma. Hypocalcemia, a
15   condition in which the serum calcium level is abnormally low, may result from a deficiency of effective PTH.

In one example, the compounds of the invention are used to manufacture diagnostic agents which are used as diagnostic
20   tools to diagnose hypercalcemia and to distinguish between-hypercalcemic conditions, i.e., to differentiate hypercalcemia mediated by PTH (e.g., hyperparathyroidism and humoral hypercalcemia of malignancy), from hypercalcemia associated with diseases which do not involve these factors (e.g., local
25   osteolytic hypercalcemia mediated by the presence of metastatic tumor cells in direct contact with bone, and certain rare types of malignancy-related hypercalcemias mediated by an increase of humoral factors, such as osteoclast activating factor (interleukin), lymphotoxin, calcitriol, type
30   E prostaglandins, and vitamin D-like sterols).

In one method of diagnosis, serum total and/or ionized calcium levels are measured by standard techniques before and after the administration of the PTH antagonists of the invention.

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PTH related hypercalcemias would be detectable as a decrease
in serum calcium levels following administration of the
antagonist of the invention. In contrast, for hypercalcemic
conditions mediated by factors other than PTH, the serum
5 calcium levels would remain unchanged even after
administration of the antagonist.

Another diagnostic application of the invention permits
measurement of the level of PTH in a biological sample in
10 order to diagnose PTH related tumors, e.g., tumors which are
associated with humoral hypercalcemia of malignancy, and for
monitoring the levels of PTH during cancer therapy. This
method involves assaying binding of the parathyroid hormone
receptor to PTH present in a tissue sample, using one of the
15 assays described herein.

A patient who is suspected of being hypercalcemic may be
treated using the compounds of the invention. Rapid
intervention is important because symptoms may appear abruptly
20 and, unless reversed, can be fatal. In one application, serum
calcium levels are stabilized by an immediate course of
treatment which includes antagonists of PTH. Such antagonists
include the compounds of the invention which have been
determined (by the assays described herein) to interfere with
25 the biological activity of PTH. To administer the antagonist,
the appropriate antibody (is used in the manufacture of a
medicament, generally by being formulated in an appropriate
carrier such as physiological saline, and administered
intravenously, at a dosage that provides adequate competition
30 for PTH binding to the PTH receptor (e.g., a dosage sufficient
to lower the serum calcium level to below 10 mg/dl). Typical
dosage would be 1 ng to 10 mg of the antibody per kg body
weight per day. Treatment may be repeated as necessary for
long term maintenance of acceptable calcium levels (i.e.,

25

levels <10.1 mg/dl). In addition, other modes of
administration can be used to administer a medicament of the
invention. This may be necessary for acute treatment of an
underlying disease condition triggering hypercalcemia; or it
5   may used, e.g., for chronic treatment of conditions such as
osteoporosis.

D.   Pharmaceutical compositions and therapeutic methods

10   The present invention further comprises pharmaceutical
compositions incorporating the compositions of the invention
and including a pharmaceutically acceptable carrier. Such
pharmaceutical compositions should contain a therapeutic or
prophylactic amount of at least one antibody identified by the
15   methods of the present invention. The pharmaceutically
acceptable carrier can be any compatible, non-toxic substance
suitable to deliver the compounds to an intended host.
Sterile water, alcohol, fats, waxes, and inert solids may be
used as the carrier. Pharmaceutically acceptable adjuvants,
20   buffering agents, dispersing agents, and the like may also be
incorporated into the pharmaceutical compositions.
Preparation of pharmaceutical conditions incorporating active
agents is well described in the medical and scientific
literature. See, for example, Remington's Pharmaceutical
25   Sciences, Mack Publishing Company, Easton, Pa., 16th Ed.,
1982, the disclosure of which is incorporated herein by
reference.

The pharmaceutical compositions just described are suitable
30   for systemic administration to the host, including both
parenteral and oral administration. The pharmaceutical
compositions will usually be administered parenterally, i.e.
subcutaneously, intramuscularly, or intravenously. Thus, the
present invention provides compositions for administration to

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a host, where the compositions comprise pharmaceutically
acceptable solution of the identified compound in an
acceptable carrier, as described above.

5   The concentration of the compound in the pharmaceutical
carrier may vary widely, i.e. from less than about 0.1% by
weight of the pharmaceutical composition to about 20% by
weight, or greater. Typical pharmaceutical composition for
intramuscular injection would be made up to contain, for
10   example, one to four ml of sterile buffered water and one µg
to one mg of the compound identified by the method of the
present invention. The typical composition for intravenous
infusion could be made up to contain 100 to 500 ml of sterile
Ringer's solution and about 1 to 100 mg of the compound.
15

Various kits comprising a releasing composition and a
detecting composition as disclosed in this invention may be
used for determining the concentration of PTH, or variants
thereof, in a sample. However, such are only examples of the
20   many possible kits which may employ the compositions of the
invention. Other kits are contemplated to be within the scope
of this invention. For example, the following patents,
articles and instruction manuals disclose assay methods which
may be adapted to include a releasing composition of the
25   present invention: U.S. Patent No. 4,935,339; No. 4,121,1978;
No. 5,232,836; No. 5,064,770; No. 5,202,266; No. 4,816,417;
No. 5,821,020 and No. 5,981,779; the disclosures of which are
incorporated in their entirety by reference herein. One
adaptation would be to replace the compositions used therein
30   with the present compositions.

In a broad embodiment, the kit of the present invention may be
adapted to be employed in an automated assay system to
determine the concentration of PTH or variants thereof. For

example, the kit of the present invention may preferably be
used in conjunction with the Nichols Advantage system.

EXAMPLES

5

Example 1

Production of antibodies that recognize and bind bioactive
hPTH

10

A.   Immunization

Human Parathyroid Hormone 1-84 was purchased from BACHEM,
California Inc., Torrance, CA.   Product No.   H-1370.1000.

15

KLH, Hemocyanin, Keyhole Limpet was purchased from Calbiochem,
La Jolla, CA.   Product No. 374805.

Glutaraldehyde was purchased from Sigma Chemical Co. St.
20   Louis, MO.   Product No. G-6247.

Human Parathyroid Hormone was coupled to KLH in molar ratios
of 200:1 as follows:

25   One milligram of PTH was solubilized in 0.20 mL 0.1 N Acetic
Acid.   The solubilized PTH was diluted to 1 mg/mL in 0.1M Na
Phosphate, 0.15 M NaCl pH 7.4.   Keyhole Limpet Hemocyanin
(KLH) was solubilized in 0.1 M Na Phosphate pH 7.2 to a
concentration 10 mg/mL by protein content.   Molar ratios by
30   protein content of 200 moles PTH to 1 mole of KLH were
calculated and the two proteins mixed at 4 degrees C.   A 10
mg/ml (1%) solution of Glutaraldehyde was prepared by diluting
the 25% aqueous solution to 1% in 0.1M Na Phosphate pH 7.2.
The mixture of 200 moles PTH to 1 mole KLH were coupled by the

28

addition of 1% glutaraldehyde to a final glutaraldehyde
concentration of 0.655 mg/mL. The gluataldehyde cross-linked
protein mixture was kept at 4 degrees C for 5-8 hours and the
reaction stopped by dilution by the addition of an equal

5   volume of 0.01 M Na Phosphate, 0.15 M NaCl pH 7.4. The total
protein concentration of the PTH-KLH was calculated on the
assumption of an 80% crosslinking efficiency.

Goats, purchased through Strategic BioSolutions, Ramona, CA,
10   were boarded and immunized as follows:

For the first immunization, goats were immunized with 2 ml of
0.1 mg of PTH-KLH solubilized with an equal volume of Complete
Freund's Adjuvant. One mL with 0.1 mg PTH-KLH with one mL
15   Complete Freund's Adjuvant. The animals were injected
intradermally on the animals at multiple sites.

Subsequent boosts were performed on a 3-4 week bases (monthly)
with 2 mL of 0.05 mg of PTH-KLH, prepared as above, in an
20   equal volume of Incomplete Freund's and injected
intradermally, as before, on the animal's side. At least two
to three boosts of PTH-KLH in Incomplete Freund's were
performed.

25   For all subsequent boosts, the animals were then immunized
only with 0.02 mg of pure PTH_{1-84} solubilized, as above, in
Incomplete Freund's Adjuvant for 2 mL of immunogen, and
injected as before on the animal's side intradermally. In
this manner, antibody clones to conformation epitopes are
30   boosted preferentially to internalize linear PTH sequences.

After at least 2 boosts of pure PTH_{1-84}, the animals were bled,
and off-the-clot serum was collected. The specificity and

titer were determined using Surface Plasmon Resonance (SPR) using BIAcore instrumentation.

Example 2

5

Isolation of antibodies to conformational PTH_{1-13}

Affinity columns to PTH amino acid sequences PTH_{38-84}, PTH_{13-34} and PTH_{1-13} were constructed as follows:

10

PTH_{38-84} and PTH_{13-34} were purchased through BACHEM, California, Product numbers H-4926.1000 and H-4145.1000, respectively. PTH_{1-13} was chemically synthesized by Research Genetics, Huntsville, Al.

15

Each of the respective PTH peptides were coupled to CNBr Activated Sepharose 4-B, by Pharmacia, as outlined in their procedure, with 1-2 mg of peptide to 2 mL of Sepharose 4-B.

20 Affinity purified antibody to PTH_{1-13} was prepared as follows:

Goat immune serum prepared as above, was sequentially purified on the affinity columns first to remove anti-PTH_{38-84} antibodies, then anti-PTH_{13-34} antibodies, and finally anti-PTH_{1-13}
25 13 antibodies. The serum extraction for all three affinity columns is the same:

At least 0.5 liters of goat immune serum was passed of the respective affinity columns in the order noted above. Each
30 affinity column consisting of at least 10 mg of peptide linked to Sepharose-4B.

The columns were washed extensively with 0.01 M Na Phosphate,
0.15 M NaCl pH 7.4. until the Spectrophotometric absorption at
280 nm no longer changed.

5   To remove non-specific serum proteins, the columns were then
washed with 0.1 M Na Acetate, 0.15 M NaCl pH 4.0, again
monitoring the absorption at 280 nm. Specific antibodies to
the peptide sequences were then eluted with 0.2 M Glycine pH
2.3. The low pH 2.3 elution by the week buffer glycine

10 dissociates the antibody from the covalently linked peptide to
Sepharose 4-B. The final eluted antibodies to PTH$_{1-13}$ are then
neutralized with the addition of dilute 0.1 N NaOH to bring
the pH back to 7.4.

15 The affinity purified goat anti-PTH$_{1-13}$, is then tested for
purity by HPLC analysis. Protein concentration determined by
molar extinction coefficient, absorption at 280 nm, 1.4 A^{280} =
1 mg/mL. The affinity purified antibody binding and
specificity then were determined using SPR by BIAcore

20 instrumentation. The antibody is stored at 4 degrees C, or -
70 degrees C for long term storage.

Antibody affinity purification columns consisting of hPTH$_{1-13}$,
hPTH$_{13-34}$, and hPTH$_{39-84}$ are constructed with these peptides

25 respectively liked to Sepharose 4B. The animal serum
generated in the immunization sequence can be isolated for
these respective antibodies in the following sequence. First
antibodies to hPTH$_{39-84}$ are removed form the sera, followed by
removing antibodies to hPTH$_{1-34}$. Then the final affinity column

30 of hPTH$_{1-13}$ is used to isolate the anti-hPTH$_{1-13}$ antibodies which
recognize the bioactive conformationally correct N-Terminal
sequence of hPTH. Although in our experiments the antibodies
to hPTH$_{13-34}$ and hPTH$_{39-84}$ were first removed to prove there were
no overlapping epitopes, it is possible to isolate the

bioactive hPTH$_{1-13}$ conformational antibodies without removing the other antibodies first.

EXAMPLE 3

5

Labeling of anti-PTH$_{1-13}$ antibody with acridinium

A purified anti-PTH$_{1-13}$ antibody was labeled with acridinium by the method as follows:

10

Acridinium as a "sulfonyl chloride ester" is crosslinked to the antibody of the invention by the reaction of the lysly moiety of the epsilon amino group of lysine in proteins, such as antibodies, to the acridinium ester. The reaction products

15 are separated by size exclusion chromatography on Sepharose G-25 with 0.1 M Na Phosphate, pH 6.0.

EXAMPLE 4

20

Immunometric assay for bioactive hPTH

Affinity purified antibodies to bioactive N-Terminal PTH (anti-PTH$_{1-13}$) are labeled for detection and anti-PTH$_{39-84}$

25 antibodies are labeled for capture.

Biotinylated capture antibodies (anti-PTH$_{39-84}$) are used to bind PTH from a sample. The capture antibodies recognize both full length PTH (PTH$_{1-84}$) and C-terminal fragments of PTH.

30 Acridinium labeled detection antibodies (anti-PTH$_{1-13}$) are used to bind the bioactive N-terminal epitope of PTH in a sample. The capture antibody preferably recognizes and binds bioactive PTH (e.g., PTH that has a conformationally correct N-terminal region). The capture and detection antibodies "sandwich" the

32

PTH molecules in the sample. The sandwich complex is bound to
streptavidin-coated magnetic particles. The particles are
then quantitated in a luminometer as is known in the art. The
procedures are illustrated in FIG. 1.

5

Samples are obtained from normal subjects (e.g., subjects that
are apparently healthy, ambulatory, and non-medicated. The
normal subjects have normal calcium and 25(OH) vitamin D
levels. Samples are also obtained from sera of subjects that
10 was submitted for creatine clearance, and had a creatine
clearance of less than 70 mL/min. Samples were also obtained
from subjects experiencing chronic renal failure (CRF). These
samples consisted of EDTA plasma obtained from subjects on
dialysis.

15

In a preferred embodiment of the invention, 150 µL of the
biological samples were combined with 70 µL of biotinylated
goat anti-PTH_{39-84} capture antibodies, 25 µL acridinium labeled
goat anti-PTH_{1-13} antibodies, and 15 µL streptavidin coated
20 magnetic particles. The combination was incubated at 37
degrees C for 30 minutes. The combination was washed, and
read in a luminometer.

Using an immunoradiometric assay (IRMA) for PTH_{1-84}, where the
25 anti-PTH_{1-13} is labeled for detection and anti-PTH_{39-84} is
labeled for capture, the following observations are noted.
Fragments, PTH_{1-6} and PTH_{7-13} will not inhibit the assay. PTH
fragments consisting of PTH_{1-34} strongly inhibit the assay,
where PTH fragments 2-34, 3-34, 4-34 and 5-34 inhibit the
30 assay in diminishing response as amino acids are removed from
amino acid position one on PTH_{1-34} (FIG. 4). Results with other
fragments are shown in FIG. 18. Inhibition with a PTH_{7-84}
fragment also was observed to have no effect. Indicating that

33

the conformational epitope is strongly dependent on at least full residue sequence in PTH_{1-13}.

This assay was then used in a study with clinical patient
5   samples that demonstrate the presence of PTH_{7-84} fragments. The study as shown clearly demonstrates the clinical utility of the bioactive intact N-terminal antibodies in be able to detect only the bioactive intact PTH_{1-84}. The correlation studies using the antibodies of the invention and the NID
10   Intact-PTH assay in patients with early and end-stage renal disease suggest the proportion of intact PTH (PTH_{1-84}) to PTH fragments (PTH_{7-84}) is similar (slopes of 0.71 and 0.69, respectively), but is different from the normal subjects (slope of 0.52). See FIGS. 6-8).
15

Example 5

Surface Plasmon Resonance (SPR)

20   Surface Plasmon Resonance (SPR) is a technique in which a response is measured by the change in the refractive index of light on a surface as a function of the mass of material bound to the surface. Using a commercial instrument by BIAcore, SPR has been used to measure the kinetics of noncovalent
25   interactions between antibodies and their antigens. See, for example, Malmqvist, M., Curr. Opin. Immunol. 5, 282-286, (1993); O'Shannessy, D.J., Curr. Opin. Biotechnol. 5, 65-71, (1993); Schuck, P., Curr. Opin. Biotechnol. 8, 498-502, (1997); the contents of which in their entireties are
30   incorporated by reference. The BIAcore instrument has been demonstrated to be a unique tool in the measurement of immunoreagents (Adamczyk, M. et al., Bioconjugate Chem. 10, 176-185, (1999); and Adamczyk, M., et al., Bioconjugate Chem.

8, 133-145, (1997); the contents of which in their entireties
are incorporated by reference.

5   In brief, CM5 sensor chips with covalently coupled rabbit
anti-goat Fc antibodies were used. The buffer used was
phosphate buffered saline (PBS) at a pH of 7.4 with 0.005%
surfactant P-20. The flow rate was 10 µL/min. Approximately
500 response units (RU) of the antibodies of the invention was
immobilized per run. The method generally comprised a one
10   minute injection of the detection antibody (i.e., anti-PTH$_{1-13}$
antibody) at a rate of 40 µg/mL followed by a two minute
injection of an inhibitory peptide (i.e., a PTH fragment, as
described herein) at a rate of 100 µg/mL followed by a two
minute wash followed by a one minute injection of PTH$_{1-84}$ at one
15   µg/ml. Binding of the antibody and PTH$_{1-84}$ was measured 30
seconds after the end of each injection. After each run, the
surface was regenerated with 10 mM gly-HCl for regeneration.

The results of the SPR studies demonstrate the conformational
20   specificity of the N-Terminal anti-hPTH$_{1-13}$ antibodies as
follows: In FIG. 10, PTH sequences 1-6 and 7-13 cannot
compete for the binding of the anti-hPTH$_{1-13}$ antibodies alone,
and are therefore important for antibody binding. PTH$_{1-13}$ does
inhibit binding of PTH to the antibodies of the invention.
25   The data demonstrate that the antibodies of the invention bind
to amino acid residues in both PTH$_{1-6}$, and PTH$_{7-13}$ fragments.
The intact full three-dimensional epitope consisting of at
least 13 amino acids must be present for binding.

30   FIG. 11 demonstrates that amino acid residues 1 and 2 at the
N-terminus are important for binding of the N-terminal anti-
PTH antibody. The removal of the first amino significantly
reduces the binding of the antibody because the correct
conformational structure is lost. In that regard, the

peptides PTH_{1-34} and PTH_{2-34} show the greatest inhibition of binding of PTH to the antibodies of the invention.

5   In FIG. 12, removal of residues 10-13 (especially 13) limits the binding of the N-Terminal Anti-PTH antibody. PTH peptides, PTH_{1-6}, and PTH_{1-7} do not inhibit binding of PTH_{1-84} to the antibodies of the invention, and that the inhibition of binding becomes successively more pronounced as the size of the inhibitory peptide increases from PTH_{1-10} to PTH_{1-13}.

10

In FIG. 13, N-terminal PTH peptides 1-34, 1-38 and 1-84 inhibit the binding of PTH to the N-terminal antibody more strongly than PTH 1-13 These results indicate that the PTH 1-13 peptide, although conformational, does not fold into

15   exactly the same three dimensional structure that PTH amino acids 1-13 assume when they are part of a larger PTH molecule, further demonstrating the unique conformational binding of bioactive intact N-Terminal PTH 1-13 antibodies.

20   These results together identify amino acid residues in two distinct regions of PTH (the N-Terminus region amino acids 1-2 and 10-13), which are important determinants for antibody binding. Since these regions are not juxtaposed in the linear sequence of PTH, the bioactive intact N-Terminal Anti-PTH 1-13

25   antibodies recognize a conformational (nonlinear) epitope of PTH.

Example 6

30   High pressure liquid chromatography (HPLC)

Biological samples are extracted from subjects, and are eluted with 80% acetonitrile in 0.1 % trifuoroacetic acid (TFA) The resulting concentrate sample was dried in a speed vacuum. The

dry sample was then solubilized in a minimal volume (e.g., 0.5 mL) of 0.1% TFA.

HPLC was performed on an analytical C18 column, at a flow rate
5   of 1.5 mL/min, with a linear acetonitrile gradient in 0.1% TFA, collecting 1.0 minute fractions. The concentrated fractions were than dried in a speed vacuum. The resulting fractions were subsequently solubilized in 0.7% bovine serum albumin (BSA) in phosphate buffered saline (PBS). The
10 fractions were subsequently assayed on a Nichols Institute Diagnostics (NID) Advantage with Intact PTH, the bio-intact PTH assay (i.e., the assay of the invention), and the PTH_{1-38} assay.

15 The NID Intact PTH assay recognizes PTH_{7-84}, and PTH_{1-84}. The bio-intact assay recognizes PTH_{1-84}, but does not recognize PTH_{7-84} (FIG. 3). The PTH_{1-38} assay recognizes PTH_{1-84}, PTH_{1-34}, PTH_{1-38}, but does not recognize PTH_{7-84}.

20 The results of the HPLC experiments indicate that HPLC resolves chemically synthesized PTH fragments (PTH_{1-84}, PTH_{1-34}, PTH_{1-38}, and PTH_{7-84}) (FIG. 14A). HPLC resolves PTH in patient samples primarily in two major peaks, as detected by the NID assay. The first peak corresponds to PTH_{7-84} fragments, and the
25 second peak corresponds to PTH_{1-84} (FIG. 3, 14B, 15A, and 15B). As persons skilled in the art will appreciate, the actual retention times may vary.

The assay of the invention using the antibodies of the
30 invention also permits detection of additional peaks that presumably correspond to PTH fragments that have intact amino terminal portions.

The HPLC analysis of human serum patient samples, the clinical utility of the bioactive intact N-Terminal anti-PTH$_{1-13}$ antibodies are again shown in demonstrating the specificity of the antibodies to only detect intact PTH 1-84.

5

Various publications and/or references have been cited herein, the contents of which are incorporated herein by reference.

While this invention has been described with respect to

10   various specific examples and embodiments, it is to be understood that the invention is not limited thereto and that it can be variously practiced with the scope of the following claims.

What is claimed is:

1. A method for producing antibodies to a three-dimensional epitope of a bioactive human parathyroid hormone, comprising:

    a)   immunizing an animal with the bioactive human parathyroid hormone; and

    b)   recovering antibodies from the animal;

    whereby the antibodies specifically recognize the three-dimensional structure of the bioactive human parathyroid hormone.

2. The method of claim 1, further comprising immunizing the animal with the human parathyroid hormone a second time before recovering the antibodies from the animal.

3. The method of claim 1, wherein the human parathyroid hormone is coupled to a carrier.

4. The method of claim 3, wherein the carrier is keyhole limpet hemocyanin.

5. The method of claim 1, wherein the bioactive human parathyroid hormone comprises SEQ ID NO: 1.

6. The method of claim 1 or 2, further comprising isolating the antibodies.

7. The method of claim 6, wherein the antibodies are isolated by affinity chromatography.

8. The method of claim 7, wherein the antibodies are isolated by screening the antibodies with fragments of the human parathyroid hormone linked to a solid phase.

39

9. A method for producing antibodies that recognize and bind
the bioactive, three-dimensional epitope of parathyroid
hormone, comprising

5   a) immunizing an animal with parathyroid hormone;

    b) immunizing the animal with parathyroid hormone a
        second time; and

    c) recovering the antibodies from the animal,

    whereby the antibodies recognize and bind the bioactive,

10 three-dimensional epitope of parathyroid hormone.

10. The method of claim 9, wherein the parathyroid hormone is
conjugated to a carrier.

15 11. The method of claim 10, wherein the carrier is keyhole
limpet hemocyanin.

12. The method of claim 9, wherein the parathyroid hormone is
human parathyroid hormone.

20

13. The method of claim 9, further comprising isolating the
antibodies so recovered.

14. The method of claim 13, wherein the antibodies are

25 isolated by affinity chromatography.

15. The method of claim 14, wherein the antibodies are
isolated by fragments of parathyroid hormone coupled to a
solid phase.

30

16. The method of claim 15, wherein the fragments of
parathyroid hormone are selected from the group
consisting of amino acids 1-13, 13-34, and 39-84 of SEQ
ID NO: 1.

40

17. The method of claim 15, wherein the antibodies are
     isolated by a fragment of parathyroid hormone consisting
     of amino acids 1-13 of SEQ ID NO: 1.

5

18. A method for producing antibodies that recognize and bind
     the bioactive, three-dimensional epitope of parathyroid
     hormone, comprising

     a)   immunizing an animal with parathyroid hormone,

10   wherein the parathyroid hormone comprises amino
             acids 1-84 of SEQ ID NO: 1;

     b)   immunizing the animal with parathyroid hormone a
             second time; and

     c)   recovering the antibodies from the animal,

15   whereby the antibodies recognize and bind the bioactive,
     three-dimensional epitope of parathyroid hormone.

19. The method of claim 18, further comprising isolating the
     antibodies so recovered.

20

20. The method of claim 18, wherein the bioactive three-
     dimensional epitope of parathyroid hormone consists of
     amino acids 1-13 of SEQ ID NO: 1.

25   21. A method for producing antibodies that recognize and bind
     the bioactive, three-dimensional amino terminus of
     parathyroid hormone, comprising

     a)   immunizing an animal with parathyroid hormone
             conjugated to a keyhole limpet hemocyanin, wherein

30   the parathyroid hormone comprises amino acids 1-84
             of SEQ ID NO: 1;

     b)   subsequently immunizing the animal with parathyroid
             hormone; and

     c)   recovering the antibodies from the animal,

whereby the antibodies recognize and bind the bioactive, three-dimensional amino terminus of parathyroid hormone.

22. The method of claim 21, wherein the bioactive three-dimensional amino terminus of parathyroid hormone consists of amino acids 1-13 of SEQ ID NO: 1.

23. An isolated antibody that recognizes and binds the bioactive, three-dimensional epitope of parathyroid hormone.

24. The isolated antibody of claim 23, wherein the bioactive, three dimensional epitope is the amino terminus of parathyroid hormone.

25. The isolated antibody of claim 23, wherein the parathyroid hormone is human parathyroid hormone.

26. The isolated antibody of claim 23, wherein the bioactive, three-dimensional epitope consists of amino acids 1-13 of SEQ ID NO: 1.

27. An isolated antibody recognizing a peptide comprising an amino acid sequence from Ser in the 1 position to Lys in the 13 position of SEQ ID NO: 1.

28. An isolated antibody according to claim 27 recognizing a peptide consisting of an amino acid sequence from Ser in the 1 position to Lys in the 13 position of SEQ ID NO: 1.

29. An antibody that is immunoreactive with the bioactive amino-terminal portion of human parathyroid hormone.

30. The antibody of claim 29, wherein the bioactive amino-terminal portion comprises amino acids 1-13 of SEQ ID NO: 1.

5 31. The antibody of claim 29, wherein the bioactive amino-terminal portion consists of amino acids 1-13 of SEQ ID NO: 1.

32. A therapeutic composition comprising the antibody of
10 claim 29, and a pharmaceutically-acceptable carrier.

33. The antibody of claim 29, wherein the antibody reduces adenylate cyclase activity by binding to the bioactive portion of the parathyroid hormone.

15

34. Any one of the antibodies of claims 23-33, wherein the antibody is a polyclonal antibody.

35. Any one of the antibodies of claims 23-33, wherein the
20 antibody is a monoclonal antibody.

36. Any one of the antibodies of claims 23-33, wherein the antibody is a humanized antibody.

25 37. Any one of the antibodies of claims 23-33, wherein the antibody is an antibody fragment.

38. Any one of the antibodies of claims 23-33 coupled to a detectable marker.

30

39. An antibody that specifically binds to the bioactive three-dimensional epitope of human parathyroid hormone, wherein the epitope consists of amino acids 1-13 of SEQ ID NO: 1.

43

40. A polyclonal antibody that recognizes and binds the
    bioactive three-dimensional epitope of human parathyroid
    hormone produced by a process comprising the following
5   steps:
    a) immunizing an animal with human parathyroid hormone
        linked with keyhole limpet hemocyanin;
    b) immunizing the animal with human parathyroid
        hormone; and
10 c) recovering the antibodies from the animal,
    whereby the antibodies recognize and bind the bioactive
    three-dimensional epitope of human parathyroid hormone.

41. A polyclonal antibody that recognizes and binds the
15 bioactive three-dimensional epitope of human parathyroid
    hormone produced by a process comprising the following
    steps:
    a) immunizing an animal with human parathyroid hormone
        linked with keyhole limpet hemocyanin;
20 b) immunizing the animal with human parathyroid
        hormone; and
    c) recovering the antibodies from the animal,
    whereby the antibodies recognize and bind the bioactive
    three-dimensional epitope of human parathyroid hormone,
25 and wherein the bioactive three-dimensional epitope
    consists of amino acids 1-13 of SEQ ID NO: 1.

42. An antibody selective for bioactive parathyroid hormone.

30 43. An antibody selective for bioactive parathyroid hormone,
    wherein the antibody recognizes and binds at least one of
    the first thirteen amino acids of SEQ ID NO: 1.

44. An isolated antibody that recognizes and binds the
bioactive, three-dimensional epitope of parathyroid
hormone, or a variant thereof.

5   45. An isolated antibody that recognizes and binds the
bioactive, three-dimensional epitope of parathyroid
hormone, or a fragment thereof.

46. A kit comprising an antibody that recognizes and binds
10   the bioactive, three-dimensional epitope of parathyroid
hormone.

47. The kit of claim 46, wherein the antibody is coupled with
a detectable label.

15

48. The kit of claim 46, wherein the bioactive, three-
dimensional epitope consists of amino acids 1-13 of SEQ
ID NO: 1.

20 49. The kit of claim 46, further comprising tools for
obtaining a biological sample containing parathyroid
hormone from a patient.

50. The kit of claim 47, wherein the detectable label is.
25   selected from the group consisting of chemiluminescent
markers, fluorescent markers, radioactive markers, and
enzymatic markers.

51. The kit of claim 47, wherein the detectable label is an
30   acridinium ester.

52. A method for detecting bioactive parathyroid hormone in a
sample, comprising

45

a)   exposing the sample to an antibody that recognizes
        and binds the bioactive three-dimensional epitope of
        parathyroid hormone; and

b)   detecting the antibody-hormone complex,

5   thereby detecting the bioactive parathyroid hormone in
        the sample.

53.   The method of claim 52, wherein the antibody that
        recognizes and binds the bioactive three-dimensional

10   epitope of parathyroid hormone is coupled with a
        detectable marker.

54.   The method of claim 52, further comprising exposing the
        antibody-hormone complex to another antibody that

15   recognizes and binds parathyroid hormone before step (b).

55.   A method for detecting bioactive parathyroid hormone in a
        sample, comprising

a)   exposing the sample to a capture antibody that

20   recognizes and binds the bioactive three-dimensional
                epitope of parathyroid hormone;

b)   exposing the capture antibody-hormone complex to a
        detection antibody that binds a different epitope
        than the capture antibody; and

25   b)   detecting the antibody-hormone complex,
        thereby detecting the bioactive parathyroid hormone in
        the sample.

56.   The method of claim 55, wherein the detection antibody is

30   coupled to a chemiluminescent marker.

57.   The method of claim 56, wherein the chemiluminescent
        marker is an acridinium ester.

58. The method of claim 52 or 55, wherein the sample is from a patient with hyperparathyroidism.

59. The method of claim 52 or 55, wherein the sample is from a patient with hypoparathyroidism.

60. An immunoassay comprising an antibody that recognizes and binds the bioactive three-dimensional amino terminus of human parathyroid hormone.

61. The immunoassay of claim 60, wherein the bioactive three-dimensional amino terminus consists of amino acids 1-13 of SEQ ID NO: 1.
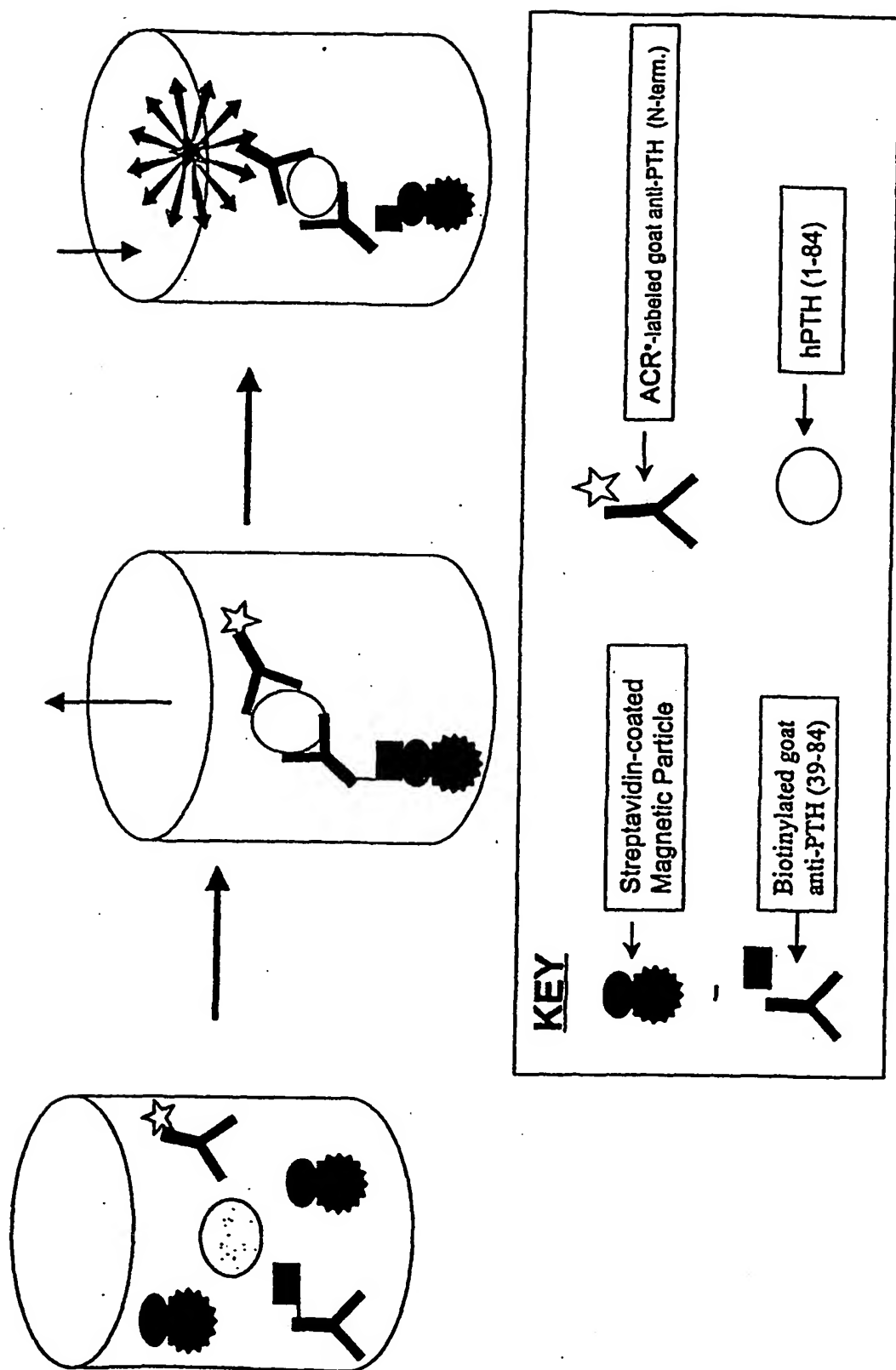
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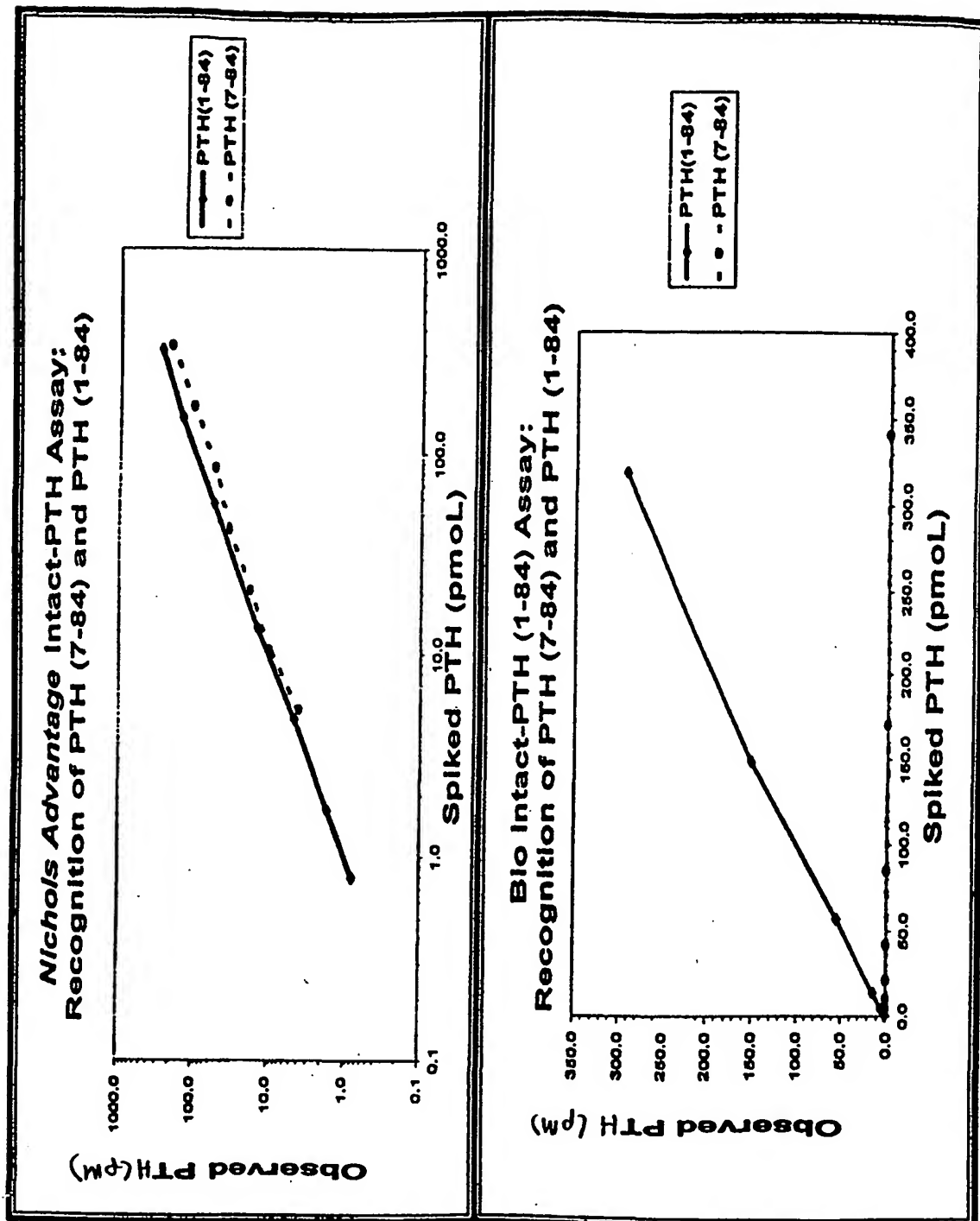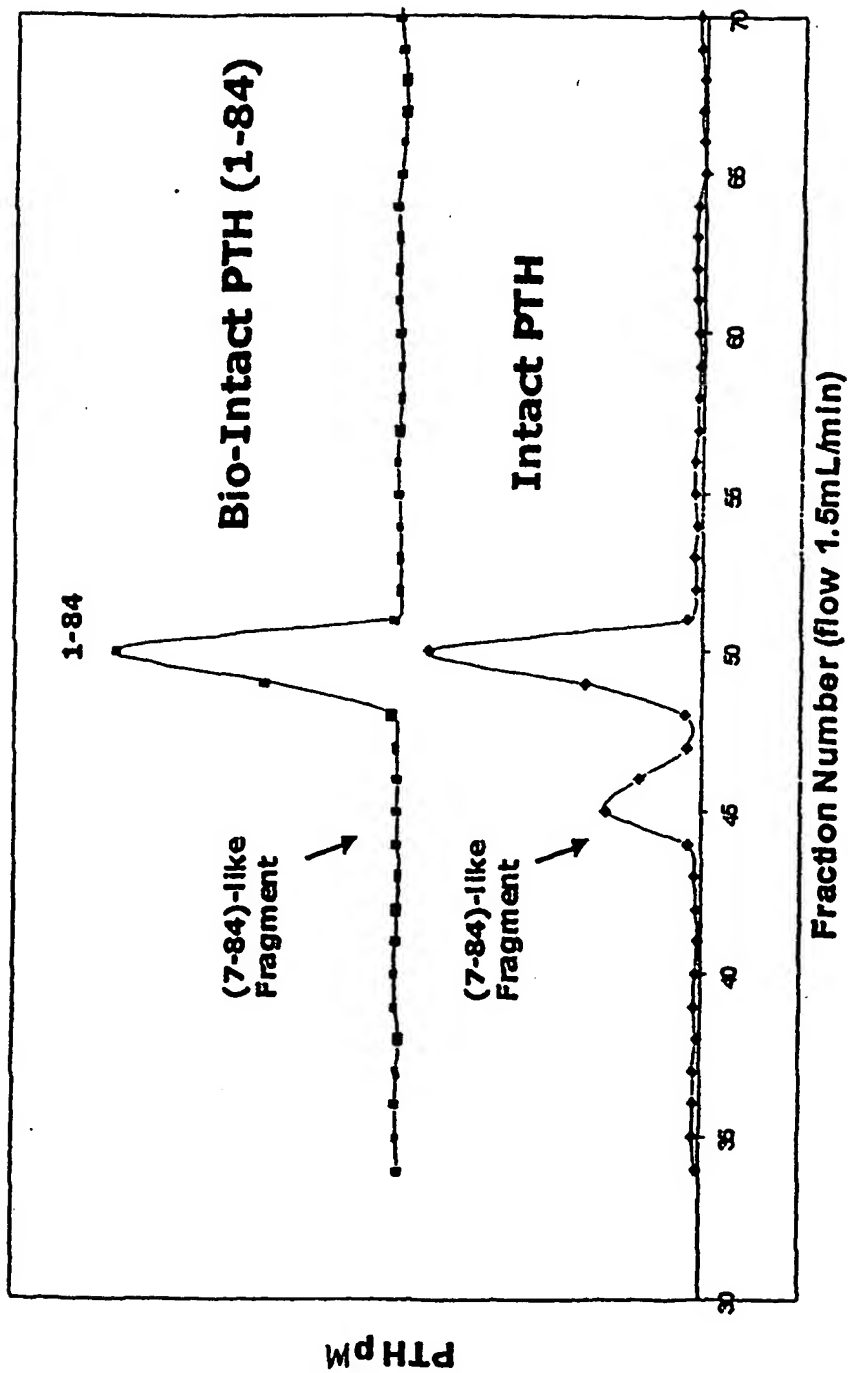
Figure 2

Figure 3

Figure 4

Figure 5

y = 0.64x + 2.34
$R^2 = 0.82$, r = 0.91

Nichols Advantage Intact-PTH (pg/mL)

Bio-Intact PTH (1-84) (pg/mL)

Figure 6

n=30
y = 0.71x - 4.64
$R^2 = 0.99$, r = 1.0

Bio Intact PTH (1-84) (pg/mL)

Nichols Advantage Intact-PTH (pg/mL)
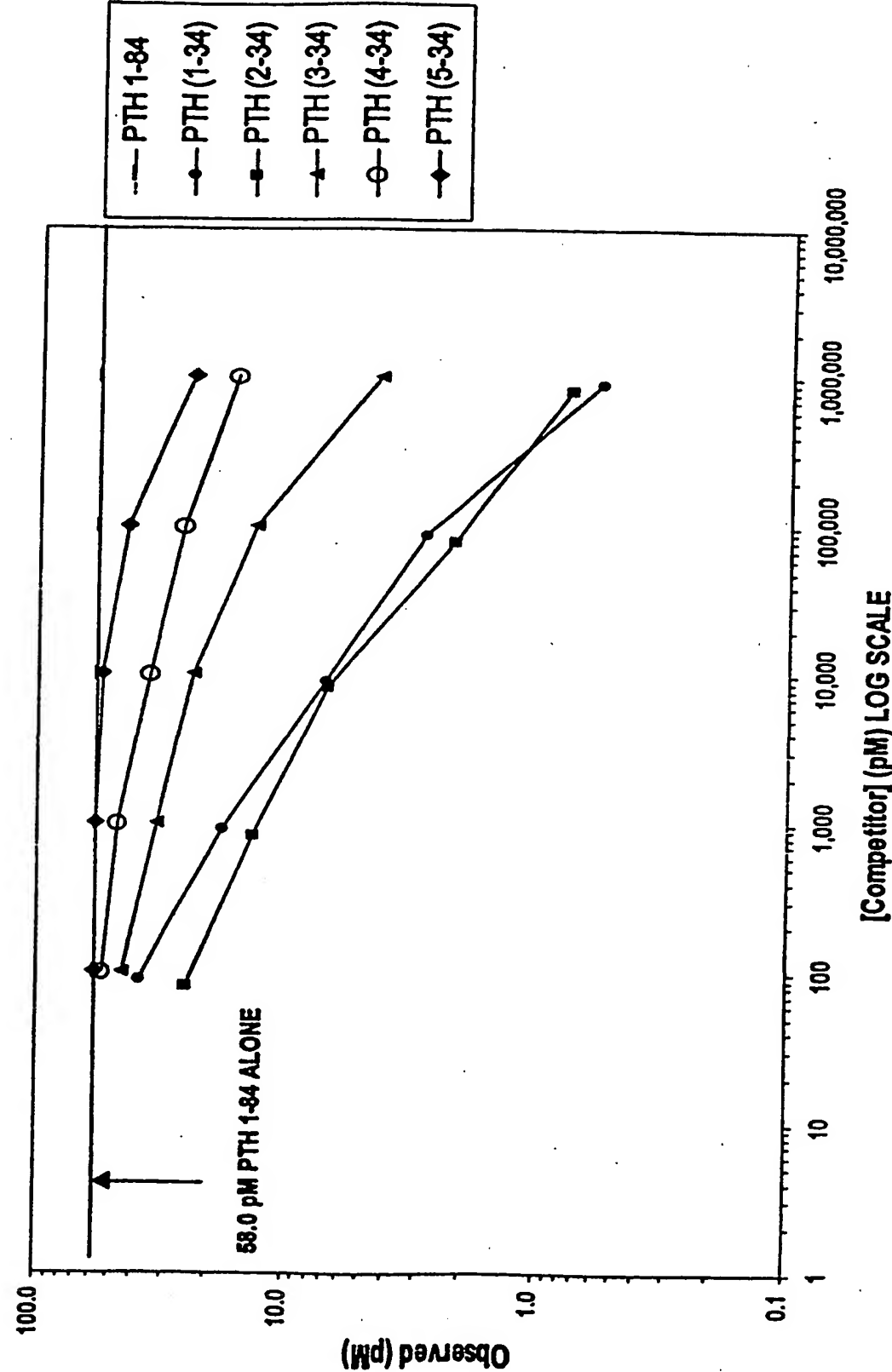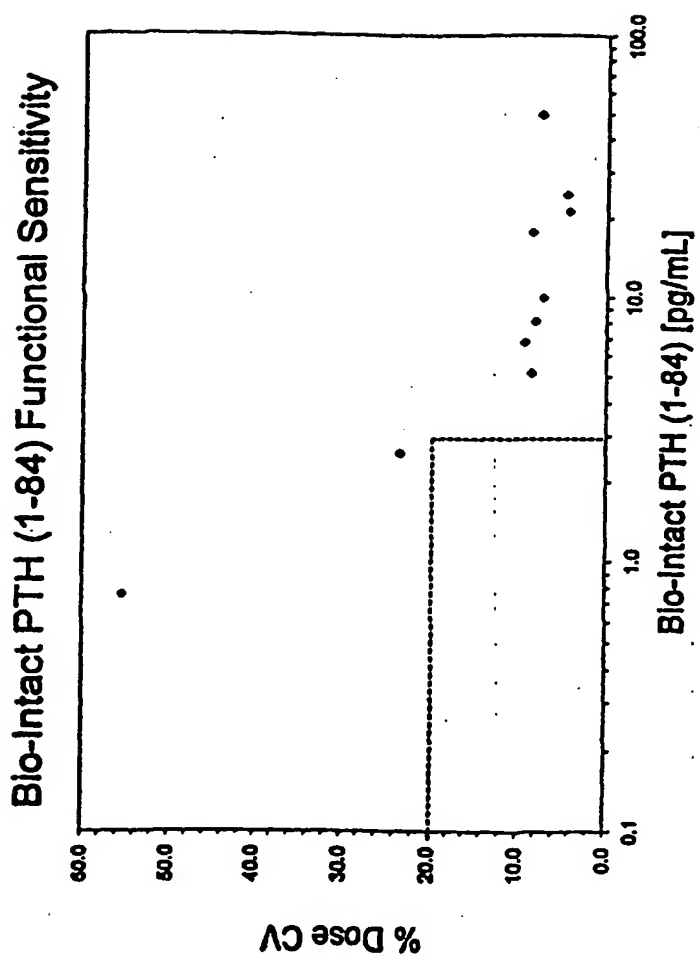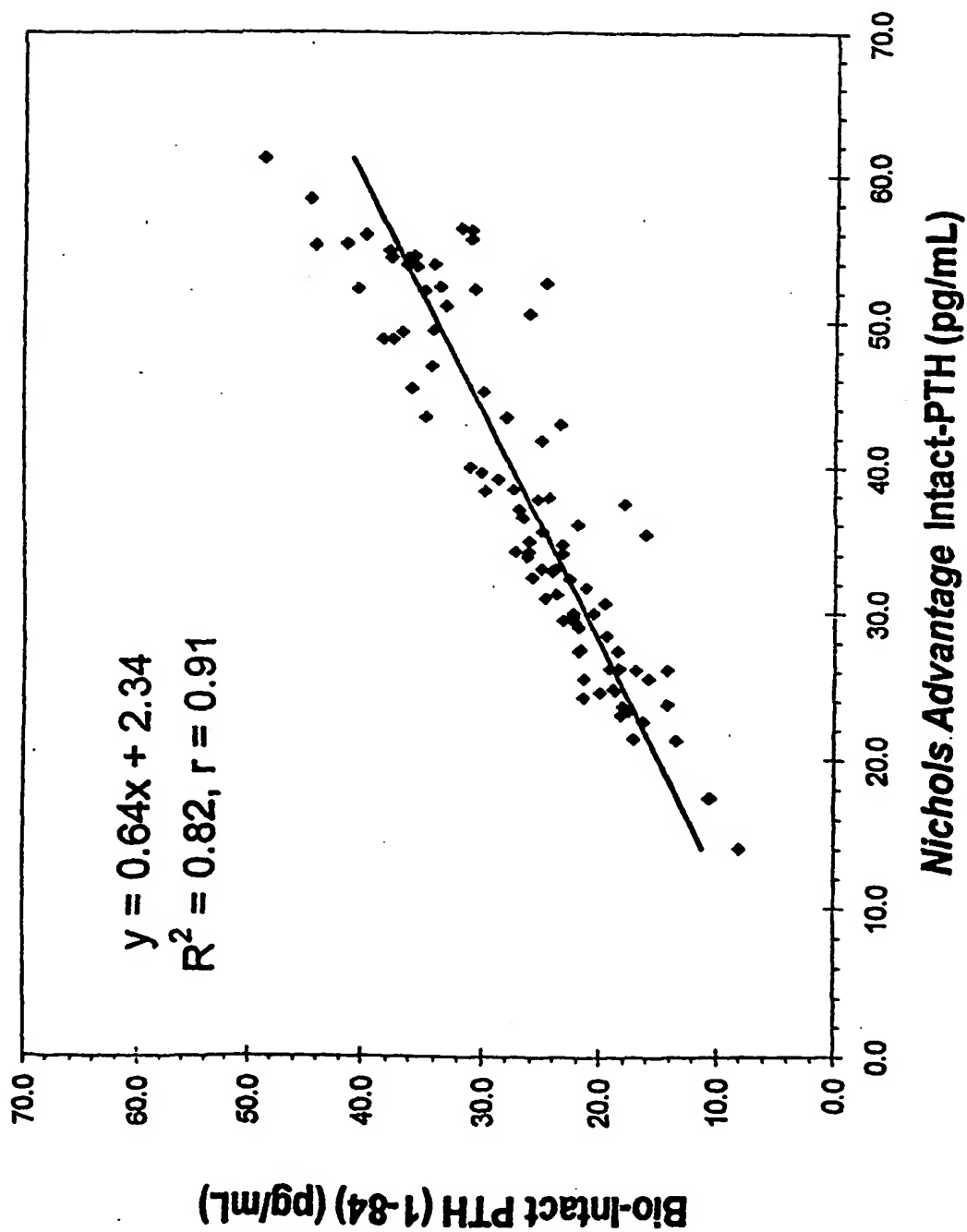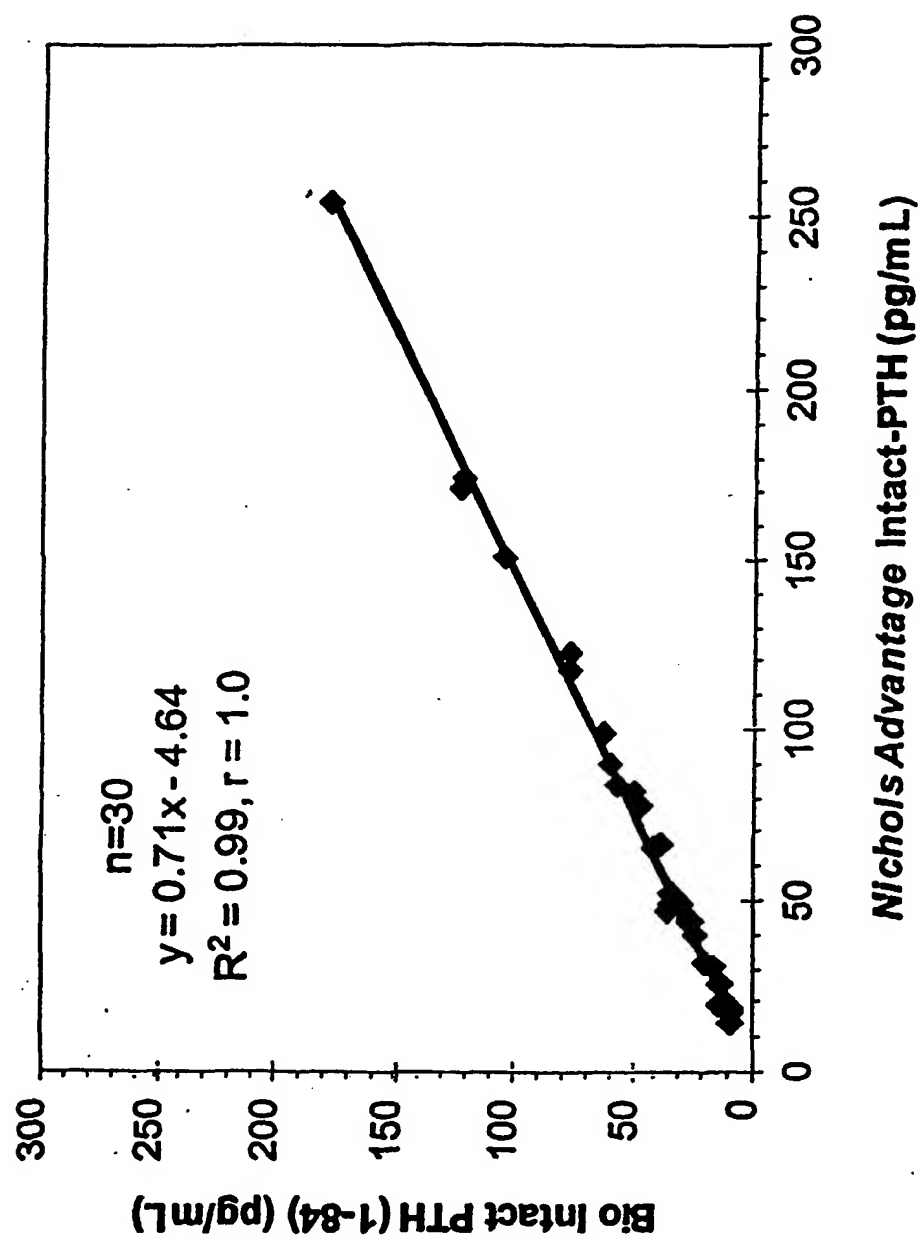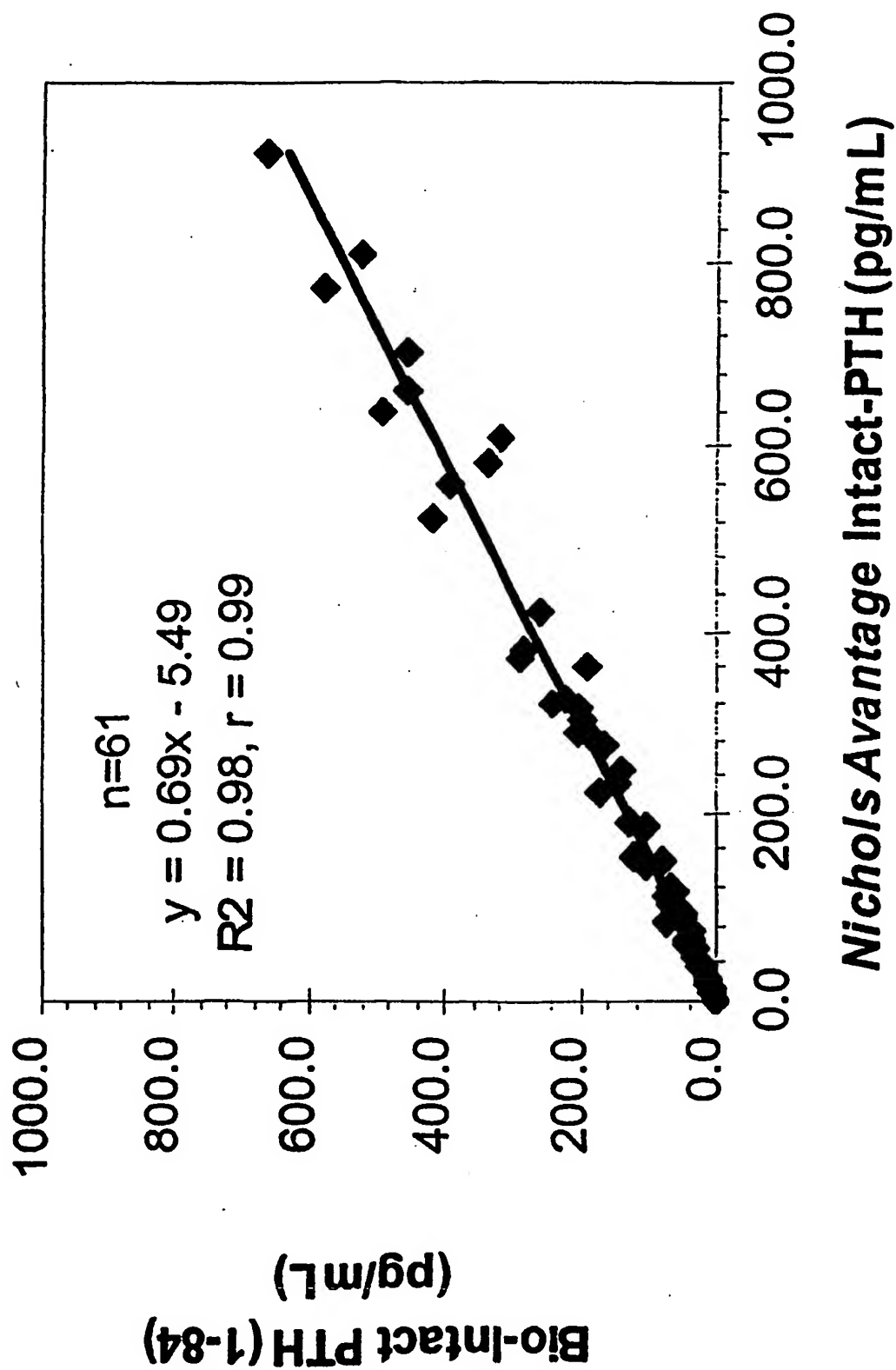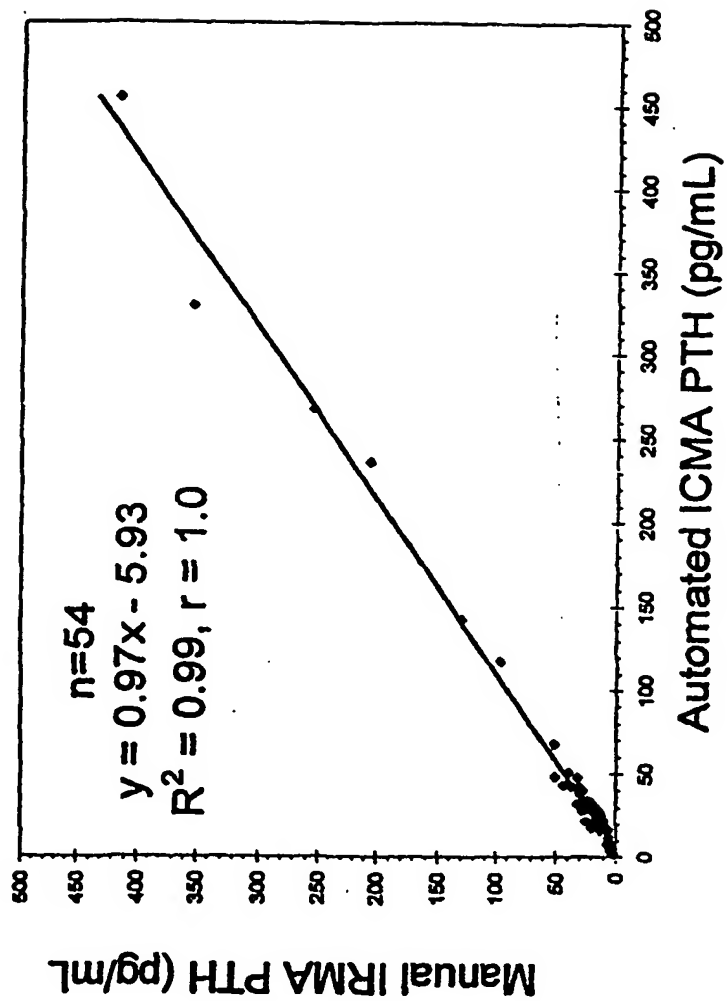
Figure 7

Figure 8

Figure 9
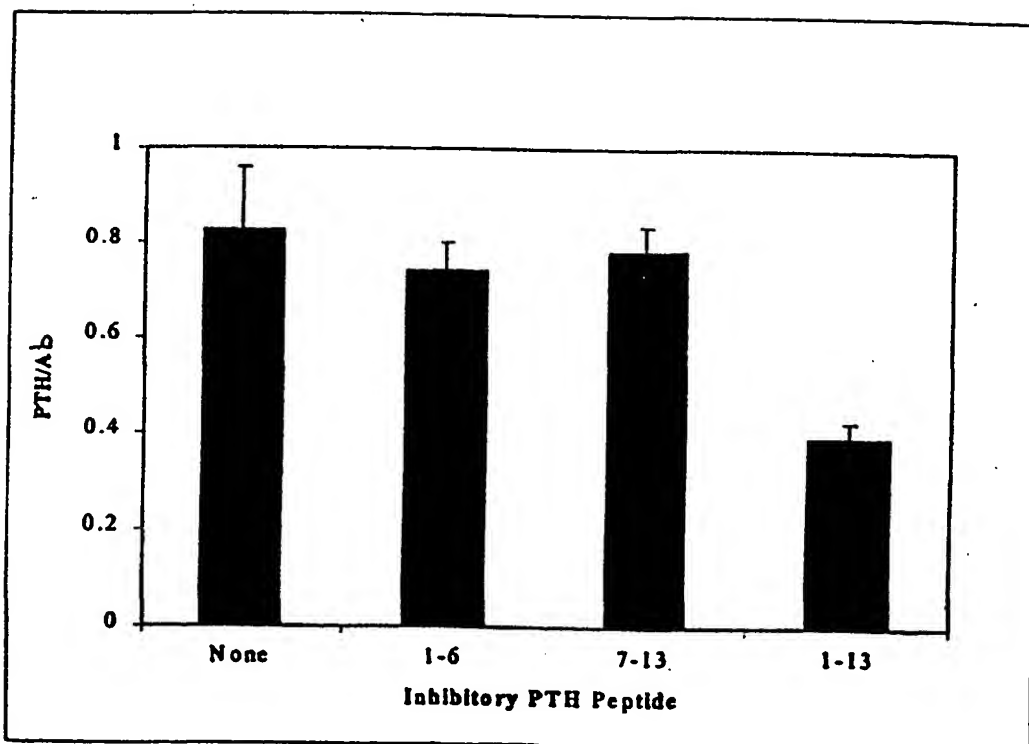
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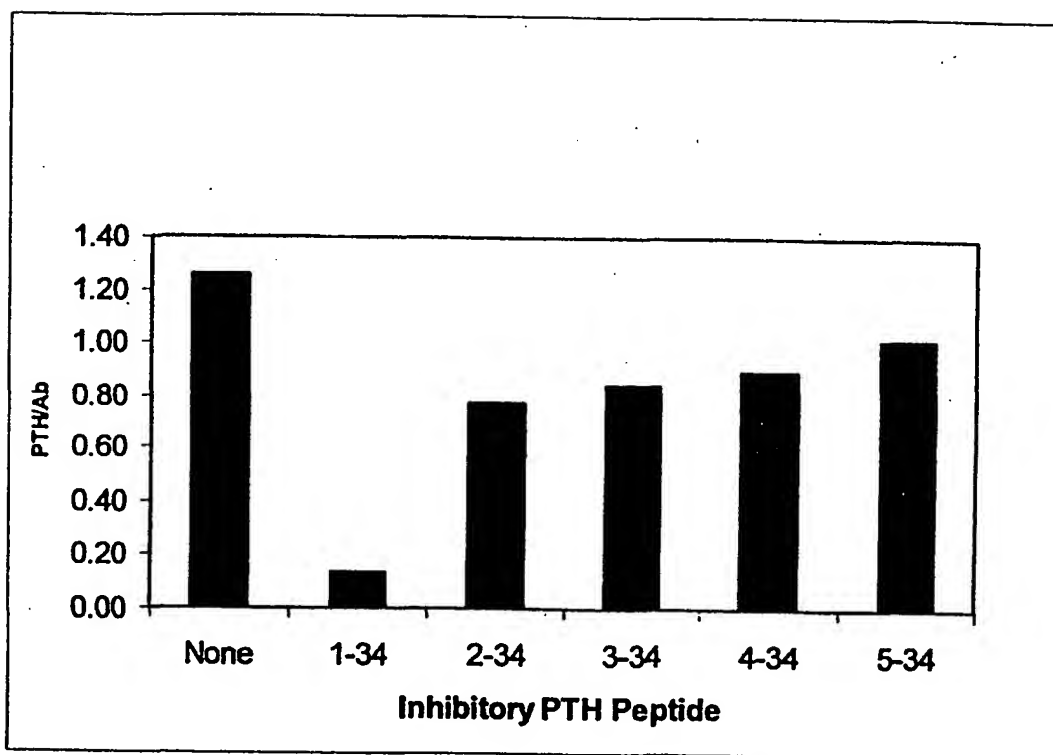
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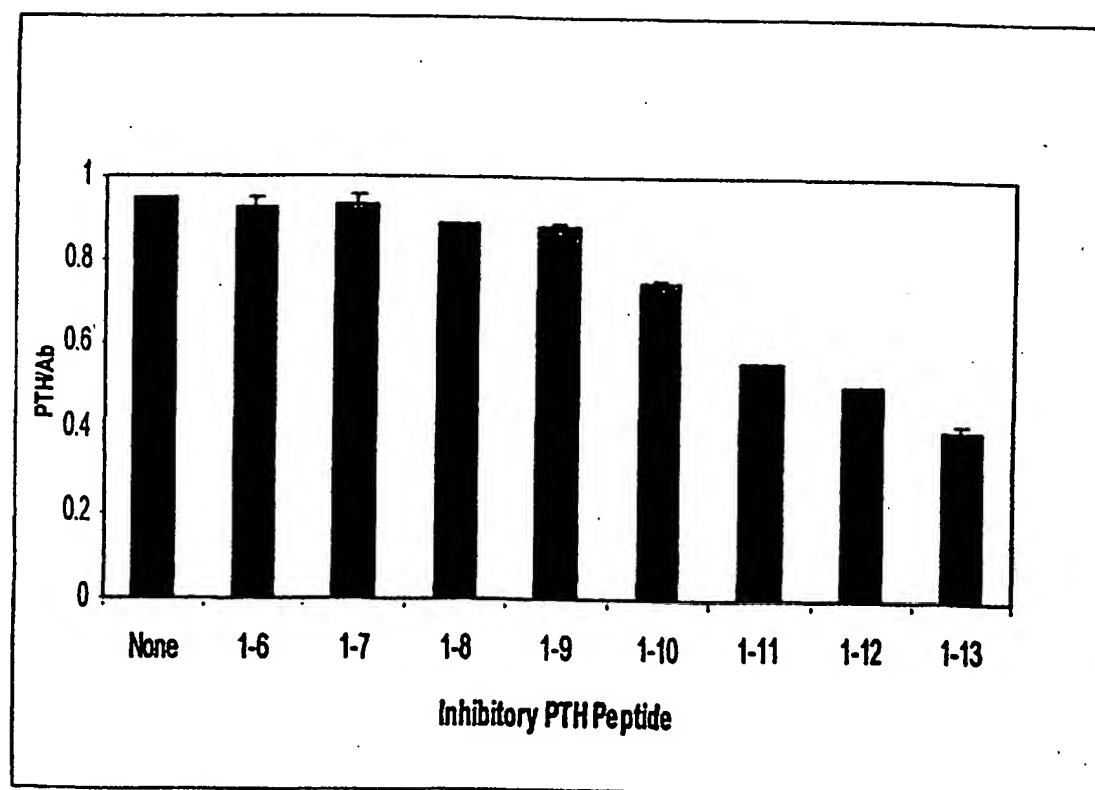
Figure 12

Figure 13

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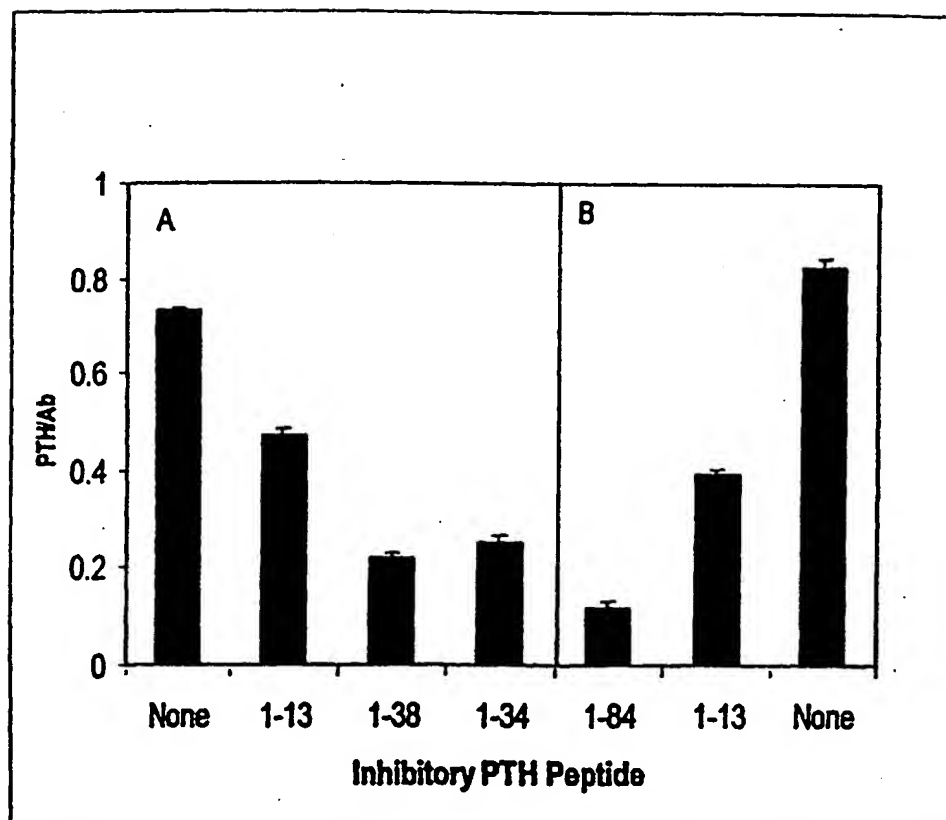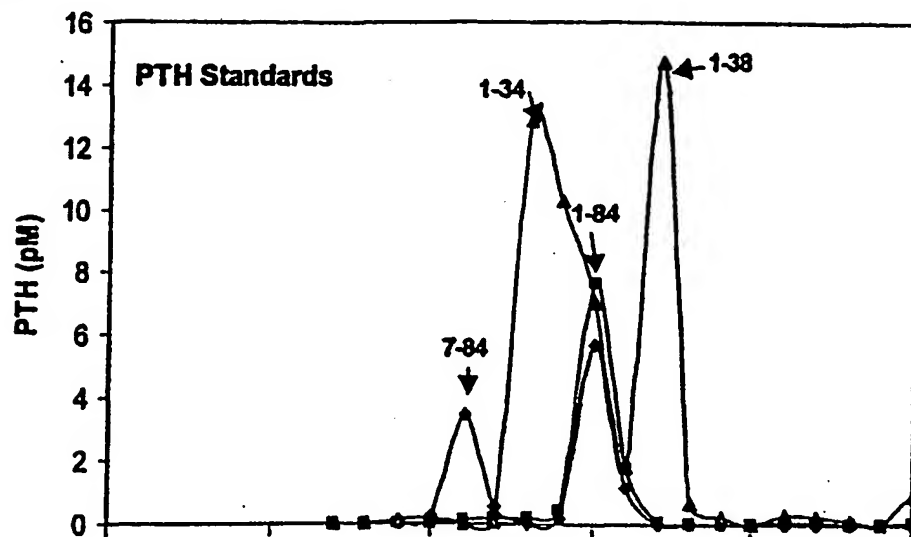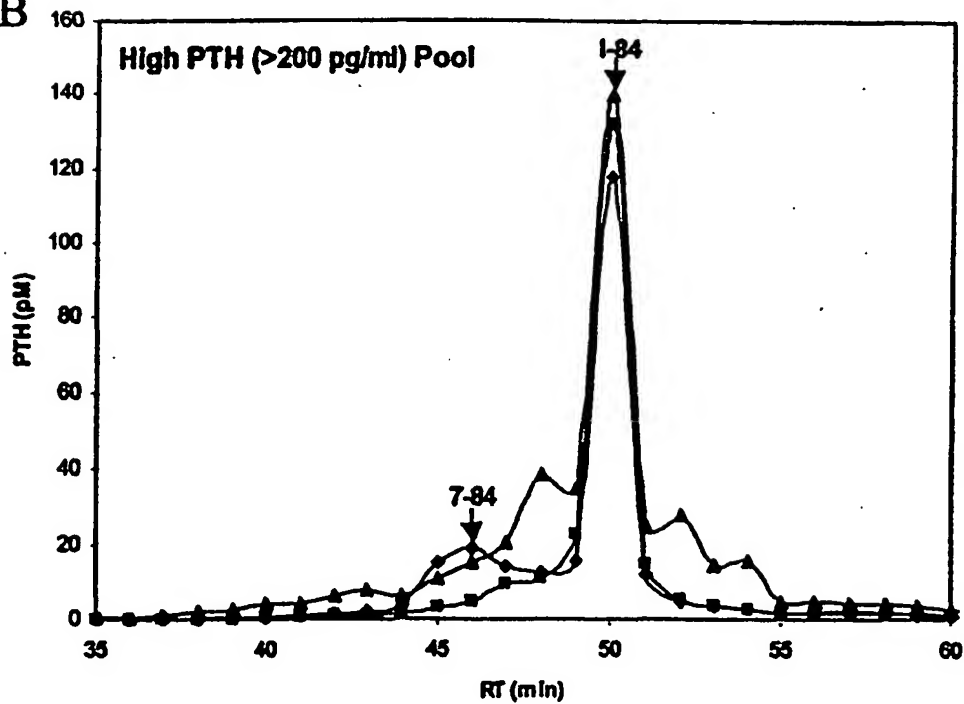
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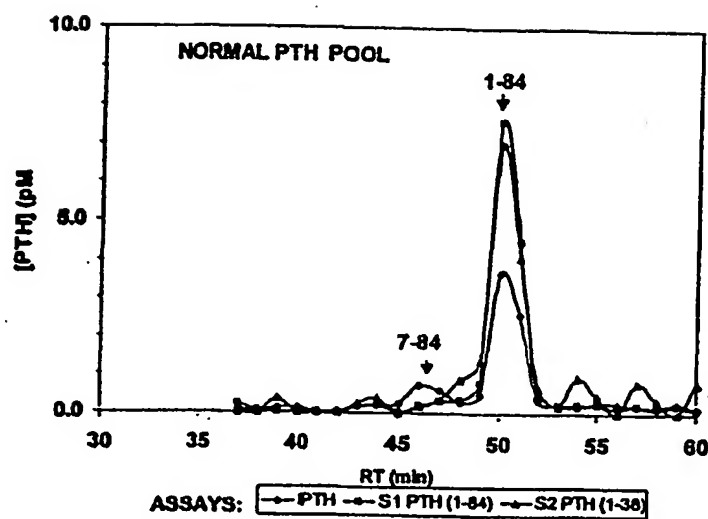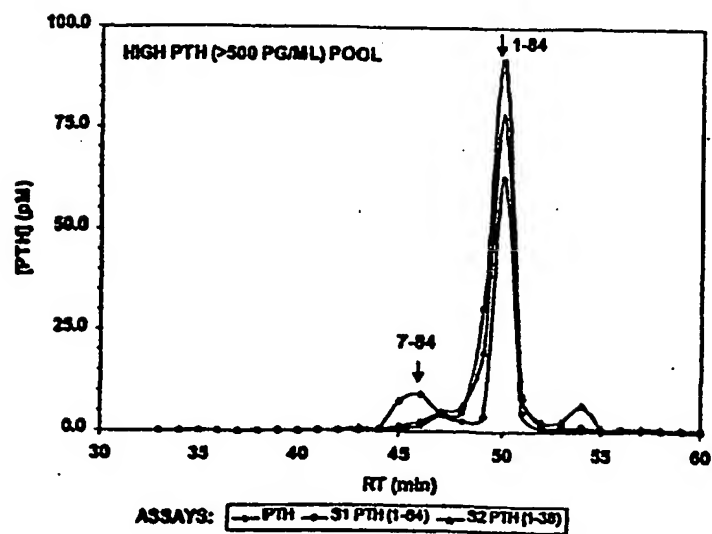
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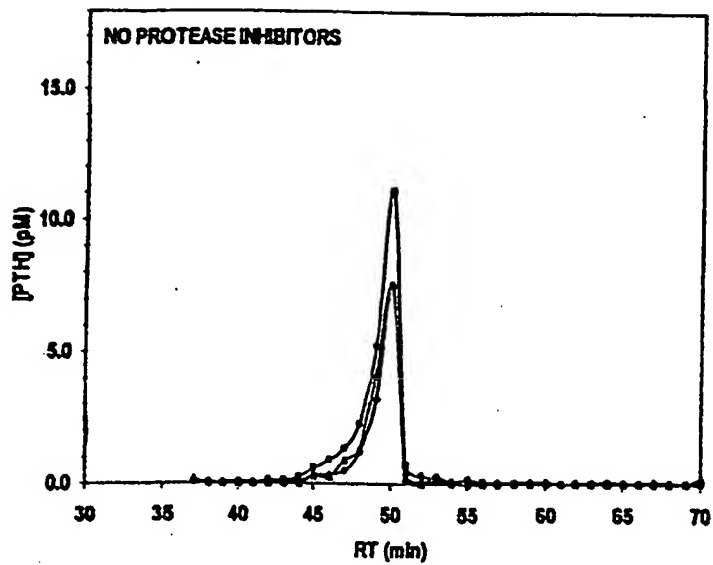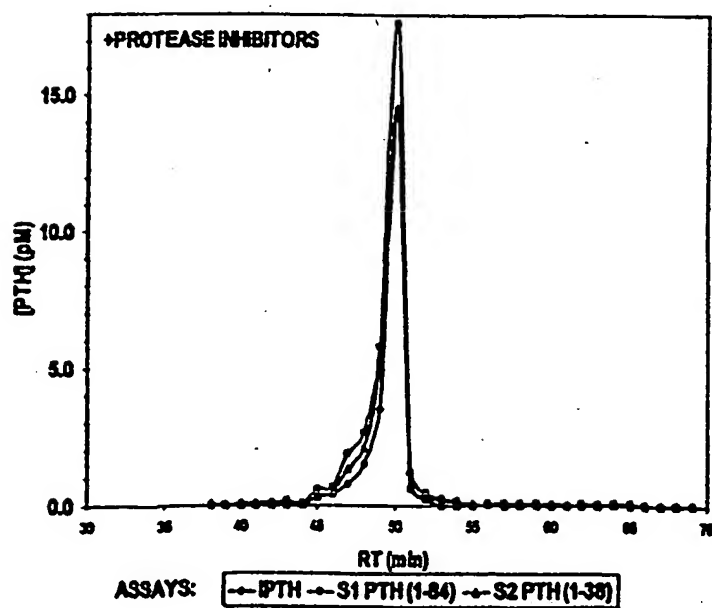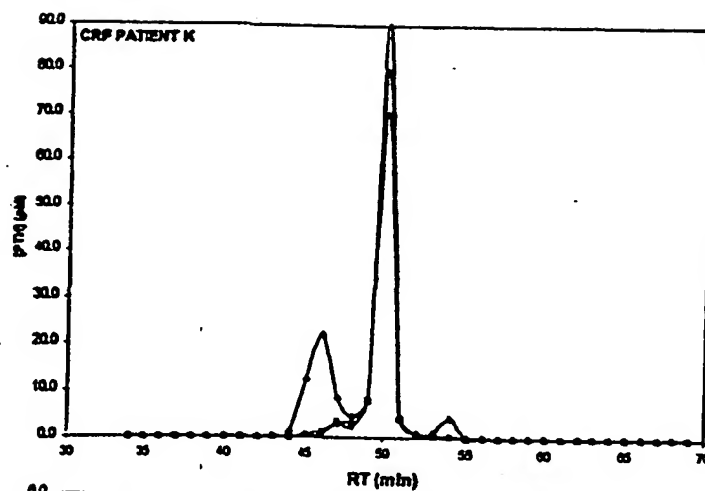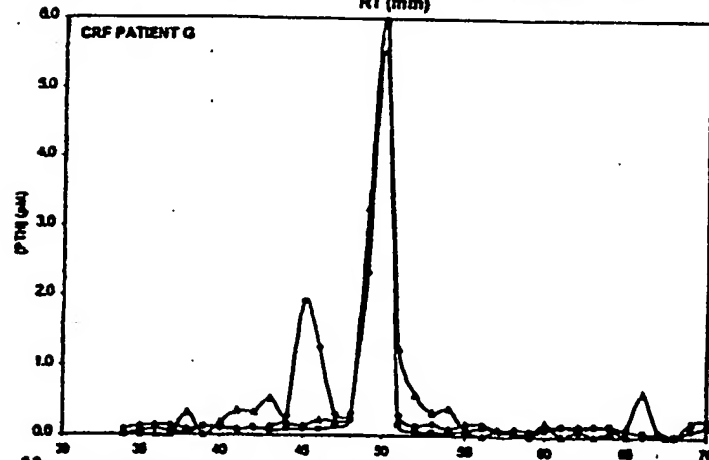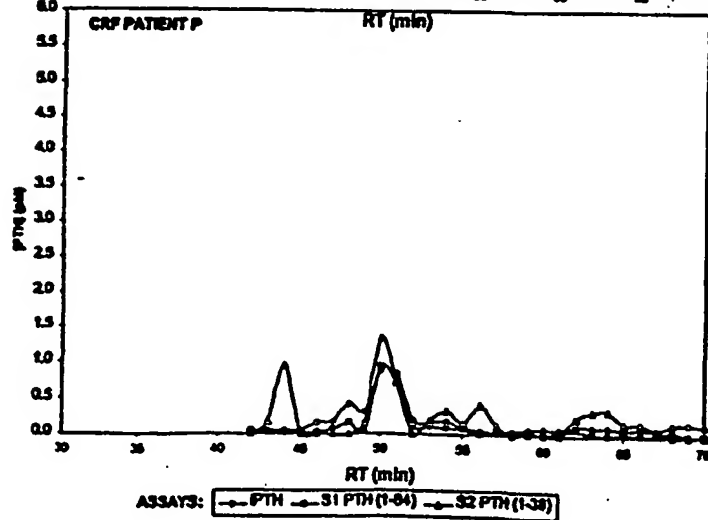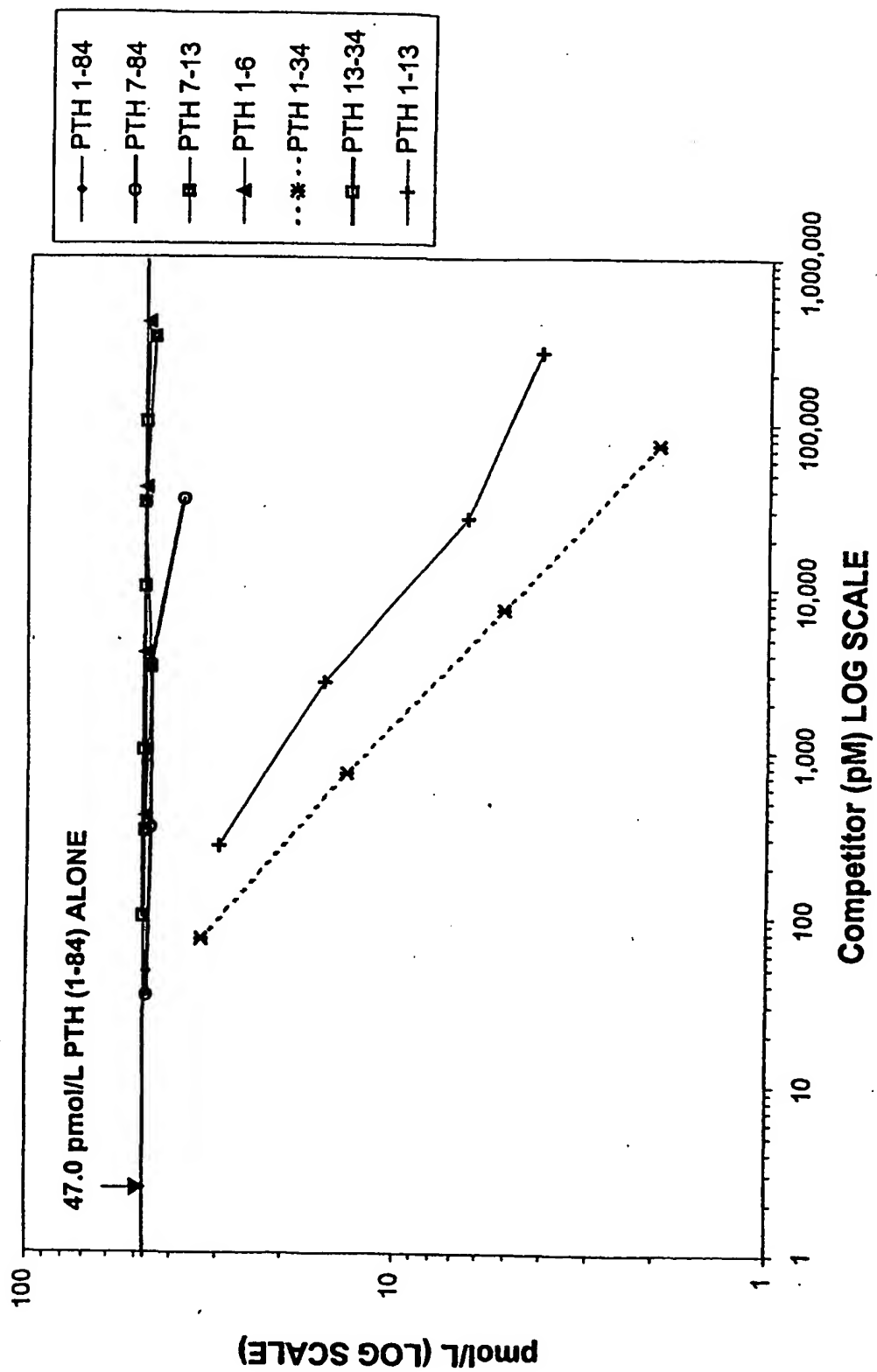
Figure 16

Figure 17

Figure 18

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<110>   Hutchison, James Scott

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